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Original Submission

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JHeimbach LLC

RECEIVED 2003

September 26, 2003

Linda S. Kahl, Ph.D.
Division of Biotechnology
and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Linda:

As discussed in our e-mails, the GRAS notices enclosed are intended to replace those I sent to you last week, on September 17.

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Ocean Nutrition Canada, Ltd., through me as its agent, herby provides notice of a claim that the food ingredient described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to foods as described to provide consumers with a supplementary source of omega-3 fatty acids in their diets.

As required, three copies of the notification are provided.

If you have any questions regarding this notification, please feel free to contact me at 202-237-8406 or jim@jheimbach.com.

Sincerely,

James T. Heimbach, Ph.D., F.A.C.N.
President

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I. GRAS EXEMPTION CLAIM

Ocean Nutrition Canada, Ltd. (ONC), through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration the use of 18/12 TG described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because ONC has determined that such use is generally recognized as safe (GRAS).

9/26/2003

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC
Agent for Ocean Nutrition Canada, Ltd.

Date

A. Name and Address of Notifier

Ocean Nutrition Canada Ltd.
757 Bedford Highway
Bedford, Nova Scotia B4A 3Z7
Canada

Contact: Janet Shay
Telephone: (902) 457-5908
Facsimile: (902) 445-2220
E-mail: jshay@ocean-nutrition.com

B. Name of GRAS Substance

The common name of the substance that is the subject of this Generally Recognized As Safe (GRAS) notification is 18/12 TG derived from fish oil by Ocean Nutrition Canada Ltd. (ONC). The crude fish oil employed in the production of 18/12 TG is extracted from multiple edible marine fish species caught off the coast of Peru. These fish species include anchovy (95–99%), sardine (1–5%), jack mackerel, Pacific mackerel, and other occasional species. Approximately 30%, on average, of 18/12 TG is composed of the two omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), primarily in the form of triacylglycerides. The total content of EPA+DHA ranges from 23 to 36%, with EPA constituting between 10–21% and DHA 8–20% of the product.

The 18/12 TG is substantially similar to other edible fish oils, as shown by the comparison of fatty acid profiles shown in Table 1. It is also substantially similar to other fish oils that are already regarded as GRAS for addition to foods, including menhaden oil (21 CFR 184.1472) and small planktivorous pelagic fish body oil (SPPFBO, GRAS Notice GRN 102; FDA 2002c). This latter fish oil is derived primarily from sardine and anchovy, the same fish that are the principal sources of 18/12 TG. Menhaden oil contains only 20-22% EPA+DHA, while SPPFBO and

18/12 TG both contain approximately 30% EPA+DHA; however, the average ratios of EPA:DHA in the three oils are similar, 1.7:1, 1.5:1, and 1.5:1 respectively.

The 18/12 TG may be sold either in the form of free oil or in microencapsulated form where the 18/12 TG constitutes approximately 55–60%, by weight, of the powder. The microencapsulated form of the product is known as Microencapsulated Fish Oil or Microencapsulated Omega-3 Fish Oil.

C. Intended Use and Consumer Exposure

ONC intends to market its 18/12 TG, in both the oil and microencapsulated forms, for addition to several categories of foods as a nutrient supplement (21 CFR 170.3(o)(20)) to increase the dietary intake of the omega-3 fatty acids EPA and DHA. The food categories proposed for addition and the proposed addition levels are listed in Table 2. These are the same food categories as are specified in the GRAS regulation for menhaden oil (21 CFR 184.1472(a)(3)), and 18/12 TG thus serves as an alternative to menhaden oil as a source of EPA and DHA. The 18/12 TG oil is proposed for addition at 67% of the use levels proposed for menhaden oil (also shown in Table 2), reflecting the average 30% EPA+DHA composition of 18/12 TG compared with 20% in menhaden oil. The 18/12 TG microcapsules are proposed for use at 120% of the use levels proposed for menhaden oil, reflecting their average 17% EPA+DHA composition. Thus, the addition rates of EPA+DHA are the same for all three fish oil products, as shown in the last column of Table 2.

It is intended that 18/12 TG will be used as the sole added source of EPA and DHA in any given food category and is not to be combined or augmented with any other source of EPA or DHA in making a food product.

On February 26, 2002, FDA issued a proposed rule (67 FR 8744) that would amend 21 CFR 184.1472(a)(3) by reallocating the uses of menhaden oil in a different set of food categories, each with a specified maximum level of use. ONC intends that any changes to the permitted uses of menhaden oil specified in 21 CFR 184.1472(a)(3) would also apply to 18/12 TG. In other words, the levels of use of 18/12 TG oil would be 67% of whatever maximum levels of use are specified in 21 CFR 184.1472(a)(3) and the levels of use of 18/12 TG microcapsules would be 120% of whatever maximum levels of use are specified in 21 CFR 184.1472(a)(3); in both cases, the permitted categories of foods would be the same. These potential future levels of use are shown in Table 3.

As with the use of menhaden oil, the maximum levels of use of 18/12 TG oil and microcapsules are designed to assure that the combined daily intake of EPA and DHA will not exceed 3 g/person/day.

The estimated mean intake of EPA and DHA combined from the proposed uses of ONC's 18/12 TG listed in Table 2 (or in Table 3) by consumers age 2 years and older does not exceed 3 g/person/day. Cumulative intake of EPA and DHA from food sources by this population, including intakes from both the proposed uses of the 18/12 TG and naturally occurring sources of fish oil, is estimated to be less than 3.1 g/person/day.

D. Basis for GRAS Determination

ONC's GRAS determination for the proposed uses of its 18/12 TG oil and microcapsules listed in Table 2 is based on scientific procedures as described under 21 CFR170.30(b).

ONC's 18/12 TG has been shown to be substantially equivalent to other edible fish oils (see Table 1), including fish oils that are already GRAS for addition to foods. The estimated intake of 18/12 TG from the intended uses specified in Table 2, in addition to intakes of EPA and DHA from natural fish oil sources, is safe and is also GRAS under the Federal Food, Drug, and Cosmetic Act (FDCA). To demonstrate that ONC's 18/12 TG is GRAS under its intended conditions of use, the safety of both whole product intake and EPA+DHA intake from consumption of ONC's 18/12 TG is established under its intended conditions of use, taking into account potential intake of EPA and DHA from natural sources in the diet. Then, this intake of the whole product and of EPA+DHA is determined to be GRAS by showing that the safety of these levels of intake is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, and is based on generally available and accepted information.

In affirming the GRAS status of menhaden oil (21 CFR 184.1472), FDA has previously derived an acceptable daily intake (ADI) for EPA+DHA of 3 g/person/day (FDA 1997b). The FDA scientists who participated in this ADI derivation are considered to be experts qualified by scientific training and experience to evaluate safe levels of exposure to EPA and DHA. A review of the scientific literature published since the date of the FDA GRAS affirmation confirms that the ADI for EPA+DHA established by the FDA is consistent with all available current information regarding the safety of consumption of EPA and DHA.

The safety of 18/12 TG intake under its intended conditions of use is evaluated through an estimate of the potential exposure to EPA and DHA from both current uses of fish oil and proposed uses of 18/12 TG, and then this cumulative estimated daily intake (EDI) is compared with the ADI established by the FDA for EPA and DHA of 3 g/person/day. If the EDI is less than or approximates the ADI, the proposed uses of 18/12 TG can be considered safe.

The cumulative EDI of EPA and DHA from consumption of 18/12 TG and from naturally occurring fish oil sources in the general population, excluding infants under the age of one year, is less than 3.1 g/person/day for the average consumer. This intake estimate reflects 100% market penetration of the proposed uses listed in Table 2 and thus is a considerable overestimate of likely consumption. This cumulative EDI of under 3.1 g/day of EPA and DHA in the general population approximates the ADI for EPA and DHA of 3 g/day established by the FDA. Therefore, 18/12 TG, in either oil or microencapsulated form, is safe under its intended conditions of use.

The publicly available data demonstrating the safety of the proposed uses of 18/12 TG oil and microcapsules was reviewed by a GRAS panel consisting of Robert G. Ackman, Ph.D., Joseph F. Borzelleca, Ph.D., and Walter H. Glinsmann, M.D. This panel evaluated the dietary exposure, source of the substance, method of manufacture, specifications, and contaminant levels, as well as information from recent published toxicological and human studies. The GRAS panel, which ONC regards as qualified by scientific training and experience to evaluate the safety of

substances added to food, concluded that 18/12 TG oil and microcapsules, meeting food grade specifications, are GRAS under their intended conditions of use.

Therefore, it is concluded, based on scientific procedures, that the intended use of ONC's 18/12 TG, as shown in Table 2, is safe and is also GRAS.

E. Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHEIMBACH LLC, 4530 Broad Branch Road, NW, Washington, DC 20008, telephone: (202) 237-8406, e-mail jim@jheimbach.com.

Table 1. Fatty Acid Profiles of 18/12 TG And Typical Edible Fish Oils				
Fatty Acid	**Percent by Weight**			
	18/12 TG	**Herring**	**Salmon**	**Sardine**
14:0	7.42	7.19	3.28	6.52
16:0	17.05	11.70	9.84	16.65
18:0	3.51	0.82	4.24	3.89
16:1 undiff	8.46	9.64	4.82	7.51
18:1 undiff	12.60	11.96	16.98	14.75
20:1 undiff.	0.00	13.62	3.86	5.99
22:1 undiff	0.00	20.61	3.38	5.59
18:2 undiff.	1.47	1.15	1.54	2.01
18:3 undiff.	1.51	0.76	1.06	1.33
18:4 undiff.	3.05	2.30	2.80	3.02
20:4 undiff.	2.08	0.29	0.68	1.76
20:5 n-3	18.55	6.27	13.02	10.14
22:5 n-3	2.40	0.62	2.99	1.97
22:6 n-3	11.85	4.21	18.23	10.66
Sources of Data: 18/12 TG: Average of 5 lots produced by ONC Herring, salmon, sardine oils: USDA Nutrient Database for Standard Reference, Release 16				

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Table 2. Maximum Levels of Use of Menhaden Oil, 18/12 TG Oil, and 18/12 TG Microcapsules and Resulting Addition Levels of EPA+DHA

Category of Food[1]	Maximum Level of Use			EPA+DHA Addition Level
	Menhaden Oil[2]	18/12 TG		
		Oil	Micro-capsules	
Cookies and crackers (1)	5.0%	3.3%	6.0%	1.0%
Breads and rolls (white and dark) (1)	1.0%	0.7%	1.2%	0.2%
Fruit pies and custard pies (1)	7.0%	4.7%	8.4%	1.4%
Cakes (1)	10.0%	6.7%	12.0%	2.0%
Cereals (4)	4.0%	2.7%	4.8%	0.8%
Fats and oils (12), not in infant formula	20.0%	13.4%	24.0%	4.0%
Yogurt (31)	4.0%	2.7%	4.8%	0.8%
Cheese products (5)	5.0%	3.3%	6.0%	1.0%
Frozen dairy products (20)	5.0%	3.3%	6.0%	1.0%
Meat products (29)	10.0%	6.7%	12.0%	2.0%
Egg products (11)	5.0%	3.3%	6.0%	1.0%
Fish products (13)	20.0%	13.4%	24.0%	4.0%
Condiments (8)	5.0%	3.3%	6.0%	1.0%
Soup mixes (40)	3.0%	2.0%	3.6%	0.6%
Snack foods (37)	5.0%	3.3%	6.0%	1.0%
Nut products (32)	5.0%	3.3%	6.0%	1.0%
Gravies and sauces (24)	5.0%	3.3%	6.0%	1.0%

1 The number in parenthesis following each food category is the paragraph listing of that food category in 21 CFR 170.3(n).

2 Food categories and maximum use levels as specified in 21 CFR 184.1472(a)(3).

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Table 3. Maximum Future Levels of Use of Menhaden Oil, 18/12 TG Oil, and 18/12 TG Microcapsules and Resulting Addition Levels of EPA+DHA Under the Proposed Rule

Category of Food*	Maximum Level of Use			EPA+DHA Addition Level
	Menhaden Oil	18/12 TG		
		Oil	Micro-capsules	
Baked goods and baking mixes (1)	5.0%	3.3%	6.0%	1.0%
Cereals (4)	4.0%	2.7%	4.8%	0.8%
Cheese products (5)	5.0%	3.3%	6.0%	1.0%
Condiments (8)	5.0%	3.3%	6.0%	1.0%
Egg products (11)	5.0%	3.3%	6.0%	1.0%
Fats and oils (12), not in infant formula	12.0%	8.0%	14.4%	2.4%
Fish products (13)	5.0%	3.3%	6.0%	1.0%
Frozen dairy desserts (20)	5.0%	3.3%	6.0%	1.0%
Gravies and sauces (24)	5.0%	3.3%	6.0%	1.0%
Meat products (29)	5.0%	3.3%	6.0%	1.0%
Milk products (31)	5.0%	3.3%	6.0%	1.0%
Nut products (32)	5.0%	3.3%	6.0%	1.0%
Snack foods (37)	5.0%	3.3%	6.0%	1.0%
Soup mixes (40)	3.0%	2.0%	3.6%	0.6%
Nonalcoholic beverages (3)	0.5%	0.3%	0.6%	0.1%
Chewing gum (6)	3.0%	2.0%	3.6%	0.6%
Confections and frostings (9)	5.0%	3.3%	6.0%	1.0%
Dairy product analogs (10)	5.0%	3.3%	6.0%	1.0%
Gelatins and puddings (22)	1.0%	0.7%	1.2%	0.2%
Pastas (23)	2.0%	1.3%	2.4%	0.4%
Hard candy (25)	10.0%	6.7%	12.0%	2.0%
Jams and jellies (28)	7.0%	4.7%	8.4%	1.4%
Plant protein products (33)	5.0%	3.3%	6.0%	1.0%
Poultry products (34)	3.0%	2.0%	3.6%	0.6%
Processed fruit juices (35)	1.0%	0.7%	1.2%	0.2%
Processed vegetable juices (36)	1.0%	0.7%	1.2%	0.2%
Soft candy (38)	4.0%	2.7%	4.8%	0.8%
White granulated sugar (41)	4.0%	2.7%	4.8%	0.8%
Sugar substitutes (42)	10.0%	6.7%	12.0%	2.0%
Sweet sauces, toppings, and syrups (43)	5.0%	3.3%	6.0%	1.0%

* The number in parenthesis following each food category is the paragraph listing of that food category in 21 CFR 170.3(n).

II. IDENTITY OF THE SUBSTANCE

A. Chemical Name

Because the 18/12 TG that is the subject of this GRAS notification is a mixture of fatty acids, no single chemical name exists for this substance. The primary components are triacylglycerides that include EPA and DHA. The 18/12 TG includes ~30% of these two fatty acids combined (with a permissible range of 23 to 36%); EPA constitutes between 10% and 21% of the oil and DHA constitutes between 8 and 20%.

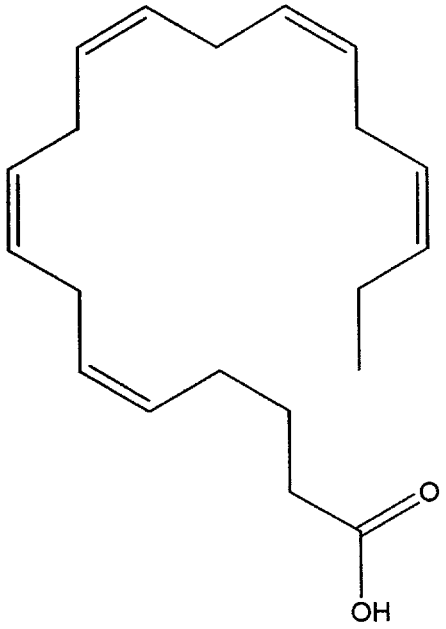
B. CAS Registry Number

Because the 18/12 TG that is the subject of this GRAS notification is a mixture of fatty acids, no Chemical Abstracts Service (CAS) Registry Number exists for this substance. The CAS Registry Numbers for EPA and DHA, the primary components of this product, are 10417-94-4 and 25167-62-8, respectively.

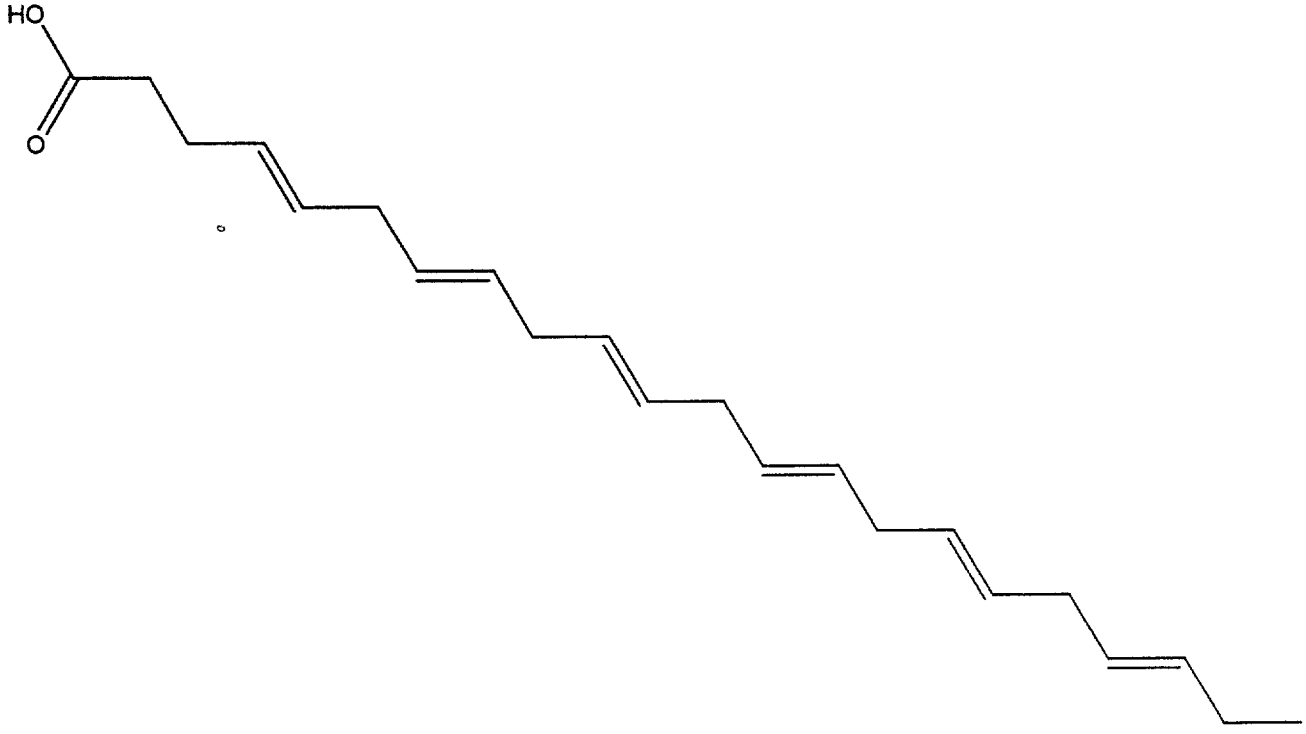
D. Molecular and Structural Formulas

Because the 18/12 TG that is the subject of this GRAS determination is a mixture of fatty acids, no single molecular or structural formula exists for this substance. The molecular formula for EPA is $C_{20}H_{30}O_2$, while the molecular formula for DHA is $C_{22}H_{32}O_2$. The structural formulas for these two major components of the 18/12 TG are shown in Figure 1.

Figure 1. Structural Formulas for EPA and DHA



Eicosapentaenoic Acid (EPA)



Docosahexaenoic Acid (DHA)

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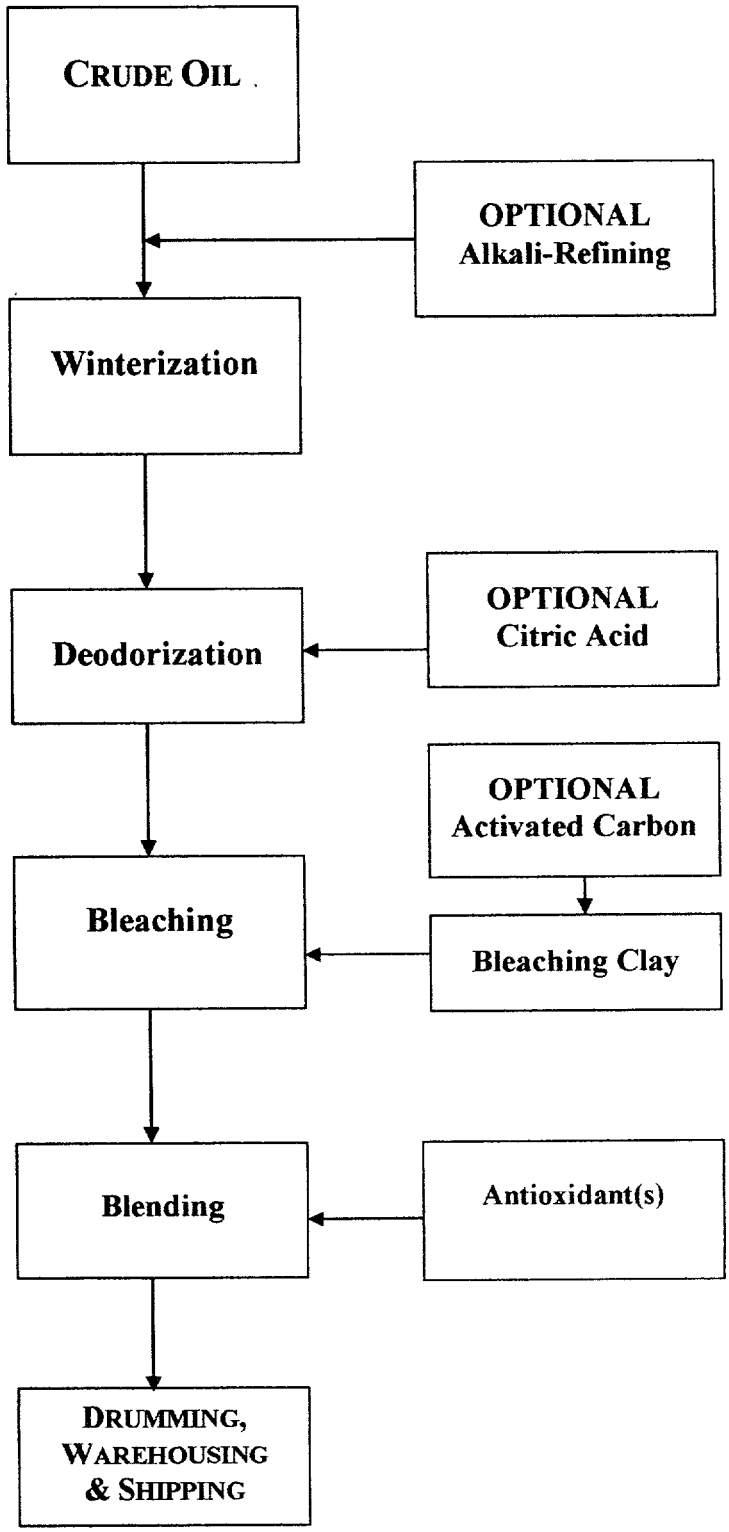
E. Source Material and Production/Purification Process for 18/12 TG

Producing 18/12 TG suitable for direct addition to foods involves processing and purifying the raw crude fish oil received from ONC's supplier. This production and purification process involves four or five basic steps:

1) (OPTIONAL) Alkali-refining
2) Winterization
3) Deodorization, either steam or wiped-film-evaporator
4) Bleaching with bleaching clay, with activated carbon optionally added
5) Blending with the addition of mixed natural tocopherols or other antioxidants

A generalized schematic of the production/purification process is shown in Figure 2. The processing steps do not necessarily occur in the order shown and, if needed, some of them (such as winterization) may be repeated. Each of these steps is discussed in greater detail below after characterization of the raw material employed in this process, crude bulk fish oil.

Figure 2. Generalized Schematic of the Production Process for 18/12 TG



1. Raw Material Specifications

The crude bulk fish oil that is the primary raw material for the production/purification process is a mixture of fatty acids (as triacylglycerides) extracted from multiple edible marine fish species caught off the coast of Peru. These fish species include anchovy (95–99%), sardine (1–5%), jack mackerel, Pacific mackerel, and other occasional species. ONC has established the following raw material specifications for the crude bulk fish oil it purchases from its supplier:

- Appearance: An amber-colored, free-flowing oil
- Odor: Characteristic of fish oil (i.e., natural fish smell)
- EPA content: 10% to 21%
- DHA content: 8% to 20%
- EPA+DHA content: 23% to 36%
- Gadoleic acid content: Not more than (NMT) 2%
- Cetoleic and erucic acid content: NMT 2%
- Acid value: NMT 10 mg KOH/g
- Peroxide value: NMT 20 meq/kg
- Anisidine value: NMT 25
- Totox number: NMT 65 (anisidine value + [2 X peroxide value])
- Arsenic: NMT 10 ppm
- Lead: NMT 0.1 ppm
- Cadmium: NMT 0.1 ppm
- Mercury: NMT 0.01 ppm

2. Alkali Refining (Optional)

In this optional process, commonly referred to as neutralization, an alkali, either NaOH or KOH, is added to the crude or winterized oil in the presence of heat to react with the free fatty acids to form soaps, which are then centrifuged out. Three water washes assure that the neutralization is complete. Both NaOH and KOH are affirmed GRAS for this process under 21 CFR 184.1763 and 184.1631, respectively, limited only by current good manufacturing practices (cGMP). This step may be performed by the supplier of the crude bulk oil prior to the product reaching the processing facilities of ONC.

3. Winterization

During winterization, also referred to as cold filtration, the oil is cooled to 0°C to crystallize the higher melting triacylglycerides; it is then passed through a filter press, while maintaining a temperature of not more than 5°C.

4. Deodorization

Deodorization may be accomplished by either of two methods, a continuous-flow process or a batch process. In the continuous flow method, crude bulk fish oil (which may have been alkali refined and/or winterized) is passed under high vacuum and at an elevated temperature through a wiped film evaporator (WFE) at a rate of up to 500 kg/hour. In the batch process, steam

deodorization, the temperature of the oil is raised to 195°C under full vacuum, after which steam is injected into the oil to remove the undesirable components.

In both processing methods, the high vacuum and elevated temperature remove polycyclic aromatic hydrocarbons (PAHs), PCBs, dioxins, furans, free fatty acids, and some sterols. The distillate is considered waste, while the residue—deodorized oil—enters the next step in the process.

5. Bleaching

Various methods of processing oils can cause darkening of the oil. Therefore, so-called "bleaching" is commonly employed to lighten the color of fish oils and other oils. Oils can be bleached by both physical and chemical means, but the most commonly used method for marine oils is a physical process—adsorption of the coloring matter using an activated earth or clay. Another physical method for bleaching, less commonly practiced, is heating the oil under vacuum (Bailey 1952). In the final step of ONC's refining process, both physical methods of bleaching described above are employed.

6. Blending and Addition of Antioxidants

As the last step in processing, the winterized, deodorized, bleached oil is blended with mixed tocopherols or other appropriate antioxidants and agitated to assure proper mixing.

F. Product Characteristics of 18/12 TG Oil

1. Specifications for the Food-Grade Material

ONC has developed specifications for 18/12 TG oil to demonstrate that it is food grade. These specifications are listed below, along with the reference to the ONC standard operating procedure (SOP) for determining compliance with each specification (except for total PCBs and metals, which are analyzed by a third-party contract laboratory using accepted validated methods for these contaminants).

• Description:	Food-grade 18/12 TG is a mixture of fatty acids (as triacylglycerides) of fish oil origin, extracted from multiple edible marine fish species caught off the coast of Peru, including anchovy (95–99%), sardine (1–5%), jack mackerel, Pacific mackerel, and other occasional species, and refined with standard methods.
• Appearance:	A clear, light yellow, free-flowing oil without sediment at room temperature (21°C) that is free from foreign matter and immiscible in water (SOP 85/05/938 and SOP 85/05/949)
• Odor and taste:	Characteristic of fish oil with no trace of rancidity or other abnormalities when tested organoleptically (SOP 85/05/906)
• EPA content:	Not less than (NLT) 10% and not more than (NMT) 21% (SOP 85/05/955), or NLT 90 mg/g and NMT 190 mg/g (SOP 85/05/917)
• DHA content:	NLT 8% and NMT 20% (SOP 85/05/955), or NLT 70 mg/g and NMT 190 mg/g (SOP 85/05/917)
• EPA + DHA:	NLT 23% and NMT 36%
• Gardner Color:	NMT 6 (SOP 85/05/927)
• Acid value:	NMT 2.0 mg KOH/g (SOP 85/05/904)
• Free fatty acid:	NMT 1.0% as oleic acid (SOP 85/05/904)
• Peroxide value:	NMT 10 meq/kg (SOP 85/05/907)
• *p*-Anisidine value:	NMT 25 (SOP 85/05/922)
• Totox number:	NMT 45 (anisidine value + [2 X peroxide value])
• Moisture content:	NMT 0.1% (SOP 85/05/918)
• Total PCBs:	NMT 2.0 ppm (USEPA Method 1668A)
• Arsenic (As):	NMT 3.5 ppm
• Lead (Pb):	NMT 0.1 ppm
• Cadmium (Cd):	NMT 0.1 ppm
• Mercury (Hg):	NMT 0.01 ppm

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2. Batch Analysis Results

To demonstrate conformance with the proposed specifications listed above, ONC analyzed several batches or lots of its final (i.e., post-bleached) 18/12 TG oil. The results of these

analyses are displayed in Table 4. These results show that all four lots of the oil are in full compliance with the established specifications, and thus the production process is under control.

Table 4. Batch Analysis Results for Four Lots of 18/12 TG

Parameter	Specification	Lot Number			
		(b)(4) (b)(4)	(b)(4) (b)(4)	(b)(4) (b)(4)	(b)(4) (b)(4)
Appearance	Clear yellow oil	Clear bright yellow oil	Clear yellow oil	Clear orange oil	Clear yellow oil
Odor	Characteristic of fish oil	Fishy	Slightly linseed	Slightly linseed	Slightly linseed
Taste	Characteristic of fish oil	Fishy	Slightly linseed	Slightly linseed	Slightly linseed
EPA (area %)	10–21	18.9	18.7	18	19
EPA (mg/g)	90–190	166.8	163.96	160.24	168.2
DHA (area %)	8–20	11.1	12.0	16.5	13.1
DHA (mg/g)	70–190	103.14	113.78	153.18	119.44
EPA plus DHA (%)	23–36	30.0	30.7	34.5	32.1
Acid value (mg KOH/g)	NMT 2.0	0.37	0.65	0.56	0.28
Peroxide value (meq/kg)	NMT 10	0.3	1.49	0.9	0.6
p-Anisidine value	NMT 25	6.87	8.31	5.62	8.04
Totox number	NMT 45	7.47	11.29	7.42	9.24
Gardner color	NMT 6	5	6	6	5
Free fatty acid (%)	NMT 1.0	0.19	0.33	0.28	0.14
Moisture content (%)	NMT 0.1	0.05	0.02	0.03	0.02
Density	No specification	Not tested	0.93142	0.93010	0.92876
Cold Test	Clear at 0°C for 24 hours	Not tested	Pass	Pass	Pass
Total PCBs (ppm)	NMT 2.0	0.006104	0.010754	0.003151	0.000681
Arsenic (ppm)	NMT 3.5	<0.1	<0.1	<0.1	<0.1
Lead (ppm)	NMT 0.1	<0.1	<0.1	<0.1	<0.1
Cadmium (ppm)	NMT 0.1	<0.02	<0.02	<0.02	<0.02
Mercury (ppm)	NMT 0.01	<0.01	<0.01	<0.01	<0.01

3. Contaminants

Although specifications were not established for these compounds, analyses were conducted for the following contaminants in conjunction with the batch analyses described above: pesticide residues, dioxins, furans, PCBs, PAHs. None were present at levels of toxicological concern.

G. Production Process for Microencapsulated Fish Oil

1. Encapsulated Loading

The encapsulated loading is 18/12 TG oil, produced and meeting specifications and purity standards as described previously. The production process by which microcapsules are formed around the oil is described in the following paragraphs and pictured schematically in Figure 3.

2. Slurry Preparation

Gelatin dry powder is dispersed in cold water to form a suspension. The suspension is then warmed and stirred. Once the gelatin has been completely dissolved, the oil to be encapsulated (18/12 TG) is added and immediately homogenized to form an oil-in-water emulsion; homogenization continues until the desired average particle size is obtained.

Once the desired emulsion is achieved, pre-heated deionized water is added to reduce the gelatin concentration to an optimum level for coacervation. Agitation is adjusted to an optimum level for the increased volume and for coacervation and multicore particle formation. A prepared sodium polyphosphate solution is added to produce a partial dehydration/desolvation of the gelatin molecules at a temperature above the gelling point. The coacervation is initiated by adding phosphoric acid to the batch to reduce the pH. This causes the gelatin and polyphosphate to form coacervates and encapsulate the small emulsified oil particles. Acid addition is stopped once the desired particle size is achieved.

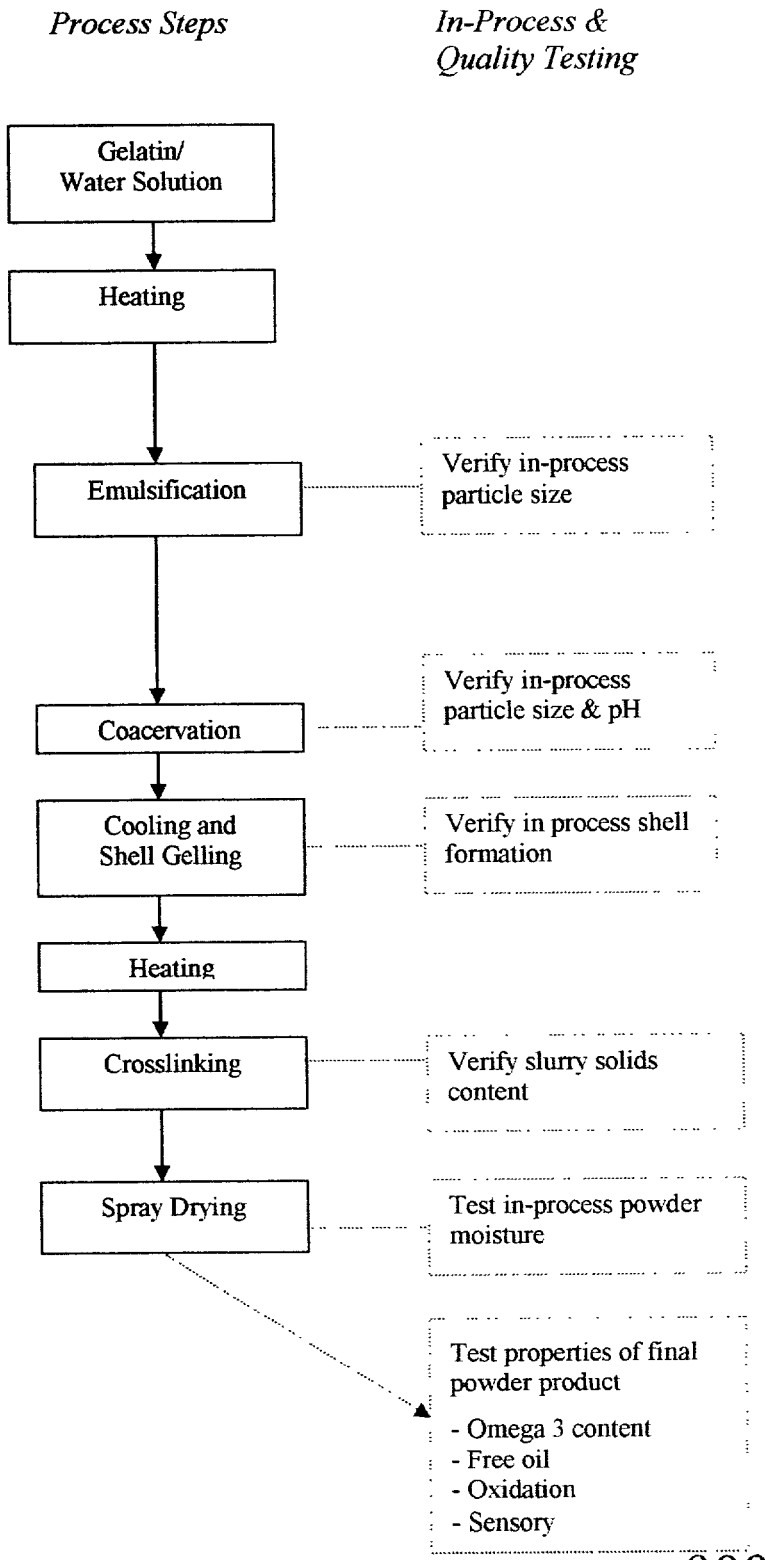
The batch is then cooled at a predetermined cooling rate so that the remaining shell materials in solution form shells around each of the microcapsules. This is continued until all free shell material (or free coacervates) has been consumed. The slurry is reheated and a cross-linking agent is added to strengthen the shell.

3. Spray Drying

The multicore microcapsule slurry is spray dried at optimum temperatures and feed rates to obtain a dry powder with desired moisture level and flow characteristics.

The product is tested to assure that it meets established specifications, including core loading, EPA and DHA content, average particle size, and maximum content of moisture and free (i.e., unmicroencapsulated) oil.

Figure 3. Flow Diagram of the Production Process for Microencapsulated Fish Oil



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H. Product Characteristics of Microencapsulated Fish Oil

1. Specifications for Food-Grade Microencapsulated Fish Oil

ONC has developed specifications for microencapsulated fish oil to demonstrate that it is food grade. These specifications are listed below, along with the reference to the ONC SOP for determining compliance with each specification (except for total PCBs, microbiological and metals, which are analyzed by a third-party contract laboratory using accepted validated methods for these contaminants).

• Appearance:	Free-flowing pale-cream colored powder (SOP 85/05/906)
• Odor and taste:	Bland odor, no fishy flavor (SOP 85/05/906)
• EPA content:	NLT 54 and NMT 114 mg/g of powder (SOP 85/05/917)
• DHA content:	NLT 42 and NMT 114 mg/g of powder (SOP 85/05/917)
• EPA+DHA content:	NLT 126 and NMT 204 mg/g of powder
• Average particle size:	NLT 35 and NMT 100 microns average (SOP 41/27/501)
• Free oil:	NMT 0.2% of powder weight (SOP 41/27/901)
• Moisture:	NMT 3.0% of powder weight (SOP 40/05/008)
• Core loading:	NLT 55% of powder weight (SOP 40/05/907)
• Sodium:	NMT 1.5% of powder weight (3rd Party Testing)
• Calcium:	NMT 0.37% of powder weight (3rd Party Testing)
• *Salmonella*:	Negative (3rd Party Testing)
• *E. coli*:	Negative (3rd Party Testing)
• *S. aureas*:	Negative (3rd Party Testing)
• Pseudomonas:	Negative (3rd Party Testing)
• Bacteria (ACC):	NMT 3000 CFU/g (3rd Party Testing)
• Yeast/mold:	NMT 300 CFU/g (3rd Party Testing)
• Coliform:	NMT 10 MPN/g (3rd Party Testing)
• Total PCBs:	NMT 0.09 ppm (3rd Party Testing)
• Arsenic (As):	NMT 3.5 ppm (3rd Party Testing)
• Lead (Pb):	NMT 0.5 ppm (3rd Party Testing)
• Mercury (Hg):	NMT 0.05 ppm (3rd Party Testing)
• Cadmium (Cd):	NMT 0.2 ppm (3rd Party Testing)

2. Batch Analysis Results

To demonstrate conformance with the proposed specifications listed above, ONC analyzed five batches or lots of microencapsulated fish oil. The results of these analyses are displayed in Table 5. These results show that all five lots of the final product are in full compliance with the established specifications, and thus the production process is under control.

Table 5. Batch Analysis Results for Five Lots of ONC's Microencapsulated Fish Oil						
Parameter	**Specification**	**Lot (b)(4)**	**Lot (b)(4)**	**Lot (b)(4)**	**Lot (b)(4)**	**Lot (b)(4)**
Appearance	Free-flowing pale-cream colored powder	Clumpy, peach	Fine, yellow	Fine, free-flowing light peach	Fine, off-white	Clumpy, yellow
Odor and Taste	Bland odor, no fishy flavor	Sweet green odor, soapy taste	Dry, slight sour odor, sour taste	Sour cream odor, sour cream taste	Slight sour cream odor, slight salty/ soapy taste	Dry, slight musty, slight sweet odor, salty, slight fishy taste
EPA content (mg/g)	54–114	91	94	96	89	92
DHA content (mg/g)	42–81	66	68	73	63	66
EPA+DHA content (mg/g)	126–204	157	162	169	152	158
Average Particle Size (microns)	35–100	56	67	55	72	61
Free Oil (%)	NMT 0.2	0.09	0.16	0.00	<0.005	0.04
Moisture (%)	NMT 3.0	1.8	2.0	2.5	2.7	2.0
Core Loading (%)	NLT 55	61	62	63	58	61
Sodium (%)	NMT 1.5	1.0	0.9%	1.2	1.1	0.91
Calcium (%)	NMT 0.37	0.038	0.01%	0.3	0.34	0.13
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative
E. coli	Negative	Negative	Negative	Negative	Negative	Negative
S. aureas	Negative	Negative	Negative	Negative	Negative	Negative
Pseudomonas	Negative	Negative	Negative	Negative	Negative	Negative
Bacteria (ACC) CFU/g	NMT 3000	<10	< 10	<10	<10	<10
Yeast/Mold CFU/g	NMT 300	<10	<10	<10	<10	<10
Coliform MPN/g	NMT 10	<3	<3	<3	<3	<3
Total PCBs (ppm)	NMT 0.09	0.02	0.02	0.02	0.02	0.02
Arsenic (ppm)	NMT 3.5	<0.1	<0.1	<0.1	<0.1	<0.1
Lead (ppm)	NMT 0.5	<0.1	<0.1	<0.1	<0.1	<0.1
Mercury (ppm)	NMT 0.05	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (ppm)	NMT 0.2	0.06	0.04	0.06	0.04	0.06

I. Analytical Method

The proposed analytical method for measuring the EPA and DHA content of marine oils and the foods to which these omega-3 fatty acids may be added is the EP 2003:1352 Method 2.4.29, slightly modified to an in-house method This method is designed to determine the fatty acid composition of marine oils in relative (area %) values, and EPA and DHA in absolute (mg/g) values using a bonded polyglycol liquid phase in a flexible fused silica capillary column. The method is applicable to the analysis of marine oils, capsules of EPA and DHA, and minor naturally occurring polyunsaturated fatty acids.

In the case of microcapsules, it is necessary to extract the oil prior to applying the method described above. The powder is physically ground with ethyl acetate in a nitrogen-flushed bowl of a planetary ball mill (Fritsch Pulverisette 6, 5 grinding balls at 400 rpm for 10 minutes). The oil is quantitatively extracted from the bowl by rinsing with hexane and filtering out the shell material. This procedure has been shown to produce accurate and reproducible results that agree closely with other techniques (enzymatic digestion of the shells, protein content, total fat analysis by acid digestion, SFE and ASE extraction methods).

III. REVIEW OF SAFETY DATA

A. Introduction

The FDA has previously reviewed the safety of consumption of fish oil containing the two omega-3 fatty acids EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS under specified conditions of use (FDA 1997b). According to the FDA, the primary safety concerns associated with excessive intakes of EPA and DHA include increased bleeding times, reduced glycemic control among diabetics, and increased levels of low-density lipoprotein (LDL) cholesterol among diabetics and hyperglycemics. ONC has expanded upon FDA's evaluation and reviewed the more recent literature to determine if more current information pertaining to these safety concerns would contradict what was concluded and recommended in the 1997 FDA opinion regarding EPA and DHA intake from fish oil. This review has focused on the safety of fish oil and of intake of EPA+DHA combined rather than on the distinct metabolic effects of EPA and DHA in isolation.

In a letter addressing omega-3 fatty acid intake and health claims regarding coronary heart disease, FDA raised the issue of whether excessive EPA and DHA intakes may exert immunosuppressive effects in humans (FDA 2000). Because of this concern raised by FDA, ONC also conducted an independent review of the published scientific literature regarding the effects of fish oils containing EPA and DHA on the immune system, with the subsequent findings and conclusions included in the following discussion.

B. Increased Bleeding Time

Evidence suggests that the intake of fish oil containing EPA and DHA may increase bleeding time, specifically by reducing platelet aggregability. Prolonged bleeding times in humans whose diets were supplemented with fish oil have been observed. However, the increases in bleeding time observed were not clinically significant; i.e., outside of the normal range for healthy adults, which is usually regarded as 1-9 minutes (Henry 1996). Therefore, uncertainty exists regarding the clinical relevance of these increased bleeding times following fish oil supplementation in the diet (Rodgers and Levin 1990). Other studies reporting increased bleeding times following daily intakes of more than 3 g/person/day of EPA and DHA used small numbers of test subjects (Atkinson et al. 1987; Harris et al. 1990; Jensen et al. 1988; Lorenz et al. 1983; Owens et al. 1990), making meaningful evaluation and interpretation of the results difficult. In addition, some of the studies that have used large numbers of healthy subjects did not observe statistically significant increases in bleeding time following daily intakes of EPA and DHA in amounts up to 3 g/person/day (Agren et al. 1990; Blonk et al. 1990; Desylpere et al. 1992; and Rogers et al. 1987).

As indicated above, some studies have shown statistically significant increases in bleeding time in subjects who consumed fish oils containing EPA and DHA (Sanders et al. 1981, 1983; Mortenson et al. 1983; Fischer and Weber, 1984; Thorngren et al. 1984; Knapp et al. 1986; Schmidt et al. 1990, 1992; Wojenski et al. 1991; Goodnight et al. 1981; Zucker et al. 1998; Harris et al. 1991). However, it should be noted that the observed increases were still within the normal range reported for healthy adults. For example, healthy volunteers who supplemented

their diet with 150 g of fatty fish per day for 12 weeks had a statistically significant increase in bleeding time compared to baseline (Thorngren et al. 1984). Similarly, healthy volunteers who consumed 1 pound/day of salmon (approximately 10 g/day of omega-3 fatty acids) had statistically significant prolonged bleeding times (Goodnight et al. 1981). In this study, the mean bleeding time prior to omega-3 fatty acid exposure was 6.75 minutes and increased to 10 minutes after 4 weeks of fish consumption. In another study, 20 healthy males consumed 4 g/day of EPA and DHA in fish oil capsules for 4 weeks (Mortensen et al. 1983). A small (16%) but statistically significant increase in bleeding time was observed relative to the control group, which received only vegetable oil. In yet another study, Harris et al. (1991) reported that daily consumption of fish oil containing 2.2 g of EPA and DHA produced a 15% increase in bleeding time that was statistically significantly elevated compared to baseline. However, this observed increase was still within the normal range of bleeding times for healthy adults.

In some studies, daily doses of fish oil resulted in prolonged bleeding times, as indicated by standard deviations of the mean bleeding times that fell outside of the baseline range, but statistical significance was not achieved. In one study (Grundt et al. 1999), healthy volunteers who supplemented their diets with fish oil containing 3.4 g EPA and DHA had increased bleeding times at the end of a 12-week study. The bleeding times increased by 30 seconds in the subjects who consumed fish oil, but this increase was not significant. In another study (Freese and Mutanen 1997), healthy subjects took fish oil capsules (containing 5.2 g of EPA and DHA) each day for 4 weeks followed by a 12-week follow-up period. There was an 18.5% increase in bleeding time in the fish oil group that returned to baseline during the follow-up period; however, no discussion or results of statistical analyses were provided.

Bleeding time data are also available from studies that have evaluated the effects of EPA and DHA intake on subjects having CHD or risk factors for CHD as their primary objective. In these studies, increased bleeding times were reported after daily intakes of EPA and DHA ranging from 3.2 to 6 g/day (Zucker et al. 1988; DeCaterina et al. 1990; Green et al. 1985; Smith et al. 1989; Schmidt et al. 1989; Solomon et al. 1990; Harris et al. 1991). However, the investigators did not discuss the clinical significance of these findings. For the most part, in studies in which fish oils were given to angioplasty or bypass surgery patients, excessive or prolonged bleeding times were not observed even though acetylsalicylic acid, a drug known to increase bleeding time, was used concurrently (Nilsen et al. 1991; Franzen et al. 1993; Bowles et al. 1991; Bairati et al. 1992; Grigg et al. 1989; Milner et al. 1989; Reis et al, 1989). In one study (Reis et al. 1989), an intake of 6 g/day of EPA and DHA in fish oils resulted in increased bleeding times in four out of 124 treated individuals (3%) as compared to controls. However, this observed increase was not statistically significant. In another study (Dehmer et al. 1988), patients who had undergone angioplasty received fish oil containing 5.4 g EPA and DHA daily for 9 months. In both the control and fish oil groups, the mean bleeding times were prolonged, but the increases were not statistically significant, and the reported bleeding times varied considerably between individuals. Heller et al. (2002) found no clinically significant differences in platelet function or coagulation after 6 days of daily administration of 86 mg/kg BW (6.0 g/70-kg person) EPA+DHA to patients recovering from major abdominal surgery. Eritsland et al. (1995) assigned 511 patients with CHD to either a fish oil group receiving 3.32 g of EPA and DHA per day, or to a control group. At the end of 9 months, bleeding time increased, but not significantly, in both the fish oil and the control groups, but the pre- and post-study bleeding time values were within the normal range. In a similar study conducted by the same group, 610 patients admitted

for coronary artery bypass grafting were given 3.32 g/day of EPA and DHA in addition to aspirin or warfarin for one year (Eritsland et al. 1996). In this study, there was a statistically significant increase in bleeding times in both groups at the end of the study compared to baseline, but there was no difference in bleeding times between the two groups.

The totality of the evidence found in the published scientific literature demonstrates that when consumption of EPA and DHA is limited to 3 g/person/day or less, there is not a significant risk for increased bleeding time above the reported normal range. However, EPA and DHA intake exceeding 3 g/day may significantly prolong bleeding times in some individuals. Currently, there are insufficient data to evaluate the clinical significance of this phenomenon in these more highly sensitive individuals.

C. Reduced Glycemic Control

Studies on non-insulin-dependent diabetics have reported increased glucose levels when 4.5 to 8 g/day of EPA and DHA were added to the diet. Previously, the FDA addressed the possible adverse effects of fish oil on glycemic control in diabetics and stated that such effects were a safety concern (FDA 1993b). Also, the FDA established, based on review of several studies, that changes in blood glucose were dependent on the amount of fish oils (i.e., EPA and DHA) consumed (FDA 1993b).

One study (Annuzzi et al. 1991) failed to observe a change in blood glucose levels among type 2 (non-insulin-dependent) diabetics who ingested 3 g/day of EPA and DHA for 2 weeks as compared to those ingesting equal quantities of other fats. In addition, in two other studies (Hendra et al. 1990; Kasim et al. 1988) in which 3 g/day of EPA and DHA were administered for 6 weeks and 3 g of EPA and DHA for 8 weeks, respectively, only transient increases in blood glucose midway through the respective study periods were observed. In another study (Borkman et al. 1989), EPA and DHA administered in the diet for 3 weeks at 3 g/person/day caused comparable increases in fasting blood glucose when either fish or safflower oil was administered, with no significant difference between groups. Vessby and Boberg (1990) examined the effect of fish oil (3 g/day of EPA and DHA) and olive oil on glucose levels. In those who consumed fish oil, there was no change in fasting glucose or glycosylated hemoglobin levels compared to baseline. However, the olive oil supplementation appeared to increase glucose levels. In a study in non-insulin dependent diabetics (McGrath et al. 1996), the results showed that fish oil supplementation (3 g/day of EPA and DHA) increased fasting glucose concentrations; however, this increase was not statistically significant. Finally, studies in type 2 diabetics (Friday et al. 1989; Glauber et al. 1988; Schectman et al. 1988; Zambon et al. 1992) reported increased glucose levels, but subjects consumed relatively high levels (4.5 to 8 g/day) of EPA and DHA.

Farmer et al. (2001) conducted a meta-analysis of 18 published randomized placebo-controlled clinical trials of the effects of fish oil supplementation in type 2 diabetes, including 823 subjects followed for a mean of 12 weeks. The doses of fish oil administered ranged from 3 to 18 g/day; the intakes of EPA+DHA were from 1.7 to 10.0 g/day. Twelve studies reported fasting glucose levels and 11 reported glycosylated hemoglobin data in ways that permitted pooling of data; none of these studies found significant changes nor was a significant effect found in the meta-analysis.

Yam et al. (2001) found no increase in glucose or evidence of hyperglycemia in hyperlipidemic subjects given 4.55 g/day EPA+DHA. In a study of hyperlipidemic patients taking simvastatin (Durrington et al. 2001), some of them also diabetic, no effect was noted on glycemic control in response to daily administration of 3.2 g EPA+DHA. Kesavulu et al (2002) found no change in glycemic control among non-insulin dependent diabetic patients receiving supplementation with 1.8 g/day EPA+DHA. Finally, in a study of obese men with dyslipidemia and insulin resistance, Chan et al. (2002) found no change in insulin resistance or in fasting blood glucose as a result of dosing with 3.36 g/day EPA+DHA in fish oil.

The FDA previously determined that EPA and DHA intakes of 3 g/person/day by diabetics exerted no clinically significant effect on glycemic control, although amounts in excess of 3 g/day may be a safety concern. Evaluation of more recent data studying the effects of fish oil containing EPA and DHA on glycemic control indicates that there is no new evidence to suggest that EPA and DHA at concentrations less than 3 g/day will adversely effect glycemic control in diabetics.

D. Increased LDL Cholesterol

The FDA noted that several studies in hypertriglyceridemic or hypercholesterolemic subjects reported increases in LDL cholesterol or apo B (apolipoprotein B, a major component of LDL) following fish oil consumption (FDA 1993b). Because elevated LDL cholesterol is a risk factor for CHD, the FDA re-evaluated these studies. As a result of this re-evaluation, the FDA found that although the available study conclusions are variable, there appears to be a trend toward increased LDL cholesterol corresponding with increased fish oil consumption in several population subgroups. The magnitude of the increase appears greater in populations with abnormal blood lipid levels, hypertension, diabetes, and cardiovascular disease. The FDA notes, however, that because the observations of increased LDL occurred in studies where large amounts of fish oils were given (resulting in EPA and DHA intakes of more than 5 g/day), any concern about changes in LDL cholesterol could be adequately addressed by limiting the intake of EPA and DHA to less than 3 g/person/day (FDA 1993b).

There has been considerable literature addressing this concern since the FDA review, but there is no indication that a change in FDA's conclusion is warranted. As discussed above, Farmer et al. (2001) conducted a meta-analysis of 18 published randomized placebo-controlled clinical trials of the effects of fish oil supplementation in type 2 diabetes. The studies included 823 subjects followed for a mean of 12 weeks, with doses of fish oil ranging from 3 to 18 g/day; the EPA+DHA intakes were from 1.7 to 10.0 g/day. Ten studies reported data on levels of LDL cholesterol. Only one study found a significant LDL-raising effect of EPA+DHA; the daily dose of EPA+DHA in this study was 6 g.

In a meta-analysis of randomized controlled trials of CHD patients, Bucher et al. (2002) reported 5 studies that studied the effects of dosing with EPA+DHA on LDL cholesterol. In 2 studies, with doses of 1.7 and 9.0 g/day EPA+DHA, LDL cholesterol increased by 7% and 5%, respectively; in 3 studies, with doses of 0.9, 4.8, and 6.9 g/day EPA+DHA, LDL cholesterol decreased by 9%, 8%, and 6%, respectively. The authors concluded that intake of n-3 fatty acids within the levels tested has little effect on LDL levels.

Donadio et al. (2001) found no change in LDL cholesterol in patients with elevated creatine levels and IgA nephropathy dosed with 3.35 g/day EPA+DHA, and a significant decrease in LDL cholesterol for those given 6.70 g/day EPA+DHA. In a study of both normotriglyceridemic and hypertriglyceridemic subjects (Mabile et al. 2001), LDL cholesterol levels did not increase after 8 weeks of dosing with 3.0 g/day EPA+DHA.

Yam et al. (2001) found no increase in total or LDL cholesterol in hyperlipidemic subjects given 4.55 g/day EPA+DHA. In a study of hyperlipidemic patients taking simvastatin (Durrington et al. 2001), some of them also diabetic, no increase occurred in LDL cholesterol among either diabetics or non-diabetics in response to daily administration of 3.2 g EPA+DHA.

Nestel et al. (2002) found no effect on either total or LDL cholesterol due to dosing dyslipidemic subjects with 3.04 g/day EPA or 2.84 g DHA + 0.52 g DPA/day. Leigh-Firbank et al. (2002) found increases in LDL cholesterol and *ex vivo* LDL oxidation and a significant decrease in the percentage of LDL cholesterol as LDL_3 in response to a daily dose of 3.0 g EPA+DHA to mildly hypertriacylglycerolemic men. Puiggros et al. (2002) administered 2.45 g/day n-3 PUFA to hypercholesterolemic patients already consuming a diet rich in olive oil; no increase occurred in LDL cholesterol, although the level of lipoperoxides in isolated LDL showed a significant increase.

In the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) study, Marchioli et al. (2002) found that administration of 1.0 g/day EPA+DHA to patients with recent myocardial infarction caused an initial rise in LDL cholesterol levels to a maximum at approximately 6 months, followed by a return to baseline that was maintained to the latest measures at 42 months. This is particularly important, because it indicates that increase in LDL cholesterol in response to administration of EPA and DHA may in some cases be a temporary, reversible effect.

This review confirms that the totality of the published literature continues to be consistent with FDA's 1997 determination that intakes of EPA+DHA of 3 g/day or less do not adversely influence levels of LDL cholesterol.

E. Immunosuppressive Effects

EPA and DHA are known to alter several aspects of the human immune response (Calder 1998; Meydani et al. 1993). Data supporting this observation come in large part from *in vitro* studies evaluating immune cell function following dietary administration of fish oils to humans. Although discrete *in vitro* assays of cell function may be relevant indicators of the potential immune response against invading pathogens, the clinical significance of these data is unknown. To determine whether fish oil consumption is clinically immunosuppressive, the overall immune status of individuals consuming EPA- and DHA-containing fish oil must be considered. However, to date no study has established a relationship between fish oil intake and an increased incidence of disease or infection. Regardless, it is often difficult to establish a meaningful relationship between increased susceptibility to disease and exposure to a certain chemical. The ability to attribute alterations in the immune system to a particular agent is made difficult by confounding factors such as lifestyle and diet, overall health status of the individual (e.g., presence of asthma or autoimmune disorders), and medications taken by the individual.

There is, however, a body of evidence suggesting that the immunosuppressive aspects of fish oil are beneficial for patients suffering from inflammatory diseases. Several studies in humans demonstrate that fish oil can ameliorate conditions of autoimmune and inflammatory diseases such as arthritis and ulcerative colitis (Kremer et al. 1987, 1995; Stenson et al. 1992; Almallah et al. 2000). In these cases, the beneficial effect of fish oil is attributed to the suppression of inflammation by reducing cellular activity and production of pro-inflammatory mediators that contribute to disease pathology.

In a double-blind, placebo-controlled study (Kremer et al. 1987), patients with rheumatoid arthritis consumed up to 6.3 g of EPA and DHA in fish oil on a daily basis for 14 weeks. Among the patients who consumed fish oil, there was a significant reduction in the number of swollen joints as compared to controls. Also, there was a significant reduction in levels of the inflammatory mediators leukotriene B4 (LTB4) and interleukin 1 (IL-1) that correlated with the reduction in swollen joints over time. In a similar study (Kremer et al. 1995), patients with rheumatoid arthritis received 9.75 g of omega-3 fatty acid supplements on a daily basis. The patients showed significant decreases in the number of tender joints, morning stiffness, and pain. Also, there was a significant decrease in circulating levels of IL-1 in patients consuming fish oil. However, there was a significant increase in the pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α). A significant increase in TNF-α was also observed in the control group. In another study, patients with ulcerative colitis received 5.4 g of EPA and DHA per day in fish oil for 16 weeks (Stenson et al. 1992). Fish oil supplementation resulted in a significant decrease in rectal dialysate levels of LTB4 and significant improvements in acute histology index and total histology index. The results of this study may, however, be due in part to the fact that the patients received concurrent treatment with prednisone. Similarly, patients with ulcerative colitis received 15 ml of fish oil extract containing 5.6 g of EPA and DHA daily for 6 months (Almallah et al. 2000). Results showed an improvement in disease activity accompanied by a decrease in serum levels of LTB4 and IL-2, and a decrease in natural killer (NK) cell activity among the patients who received fish oil. As with the Stenson et al. (1992) study, the patients maintained their existing anti-inflammatory medications during the course of the study, making it difficult to solely attribute the improvement in disease parameters to fish oil. The authors concluded that although the modest clinical improvement does not support a recommendation for fish oil supplementation as a single treatment for acute ulcerative colitis, it could be a useful co-treatment to reduce the dosage of steroids or anti-inflammatory medications required.

Researchers have also examined the effect of fish oil supplementation on other diseases mediated by inflammation, including asthma and psoriasis (Ziboh et al. 1986; Bittiner et al. 1988). The results of these studies do not provide convincing evidence that fish oil is beneficial for treatment of these diseases.

Fish oil has also been shown to decrease fever in humans, another component of the inflammatory response. Healthy volunteers who supplemented their daily diet with fish oil containing 2.71 g of EPA and DHA for 8 weeks were challenged with typhoid vaccine (Cooper et al.1993). The fish oil inhibited an expected rise in oral temperature following vaccination; however, this effect was not statistically significant relative to the control group. *In vitro* stimulation of lymphocytes obtained from the individuals who consumed fish oil showed decreased production of IL-1, a cytokine associated with the induction of fever.

One study (Kelley et al. 1998) demonstrated that DHA (in the absence of EPA) caused a decrease in the number of circulating white blood cells (WBCs) in healthy males who supplemented their diets with moderately high levels of DHA (6 g/day) for 90 days. There was a significant decrease in the number of WBCs at day 113 in the DHA group, as compared to controls. The decrease in WBCs was primarily due to a 21% decrease in the number of polymorphonuclear cells (PMNs). Although the number of PMNs was significantly decreased in the DHA group, the residual counts were still within clinically normal ranges. This same study also examined the effect of DHA on the delayed hypersensitivity response to antigen, an *in vivo* test of immune function. There was no significant difference between the DHA group and controls in their ability to elicit an antigenic response following sensitization. Also, DHA did not appear to influence IL-2 production or mitogen-stimulated lymphocyte proliferation, which was actually increased in the DHA group.

Donadio et al. (2001) administered doses of 6.70 g and 3.35 g EPA+DHA to patients with IgA nephropathy; there were no unfavorable effects on peripheral blood leukocytes. In a complex study, Thies et al. (2001a, 2001b) supplemented the diets of healthy adults age 55–75 with oils providing daily intakes of 2000 mg α-linolenic acid (ALNA), 770 mg γ-linolenic acid (GLA), 680 mg arachidonic acid (ARA), 720 mg DHA, or 1000 mg EPA+DHA (720 mg EPA + 280 mg DHA) for 12 weeks. The biological markers measured were lymphocyte proliferation; production of interleukin-2 (IL-2) and interferon-γ (IFN-γ); the number and proportion of T and B lymphocytes, helper and cytotoxic T lymphocytes, memory helper T lymphocytes, leukocytes, and NK cells in the circulation; proportion of leukocytes as total lymphocytes and proportion of lymphocytes as T lymphocytes and as NK cells; and NK cell activity. The only markers that showed significant differences among the groups, or changes from baseline, were lymphocyte proliferation (reduced by GLA and by EPA+DHA) and NK cell activity (reduced by EPA+DHA).

Turini et al. (2001) administered 25 g/day fish oil (comprising 4.3 g EPA + 2.8 g DHA) to healthy men and found no decrease in immune cell activities; on the contrary, an increase in phagocytic activity was seen in blood monocytes.

In a study of patients recovering from major abdominal surgery (Weiss et al. 2002), administration of 4.3 g/day EPA+DHA resulted in a decrease in levels of IL-6, but no change in TNF-α and an increase in monocytic human leucocyte antigen (HLA)-DR, a marker of immune competence. There was no difference between the test and control groups in the number of infections, although the n-3 fatty acid group's infections were less severe than were those that occurred in the control group. Schauder et al. (2002) also used postoperative abdominal surgery patients to study the effect of 86 mg/kg BW/day (5.8 g/day for mean=68-kg person) EPA+DHA on a variety of immune markers. No significant effects were found other than potentially beneficial increases in several markers (IL-2, IFN-γ, and TNF-α); the authors concluded that fish oil at the dosage given is not immunosuppressive in moderately stressed surgical patients.

A majority of the existing studies that have examined the effect of EPA and DHA intake on the immune system have done so by assessing discrete cellular functions in healthy human volunteers following fish oil supplementation. For the most part, daily supplementation with fish oil (EPA and DHA) resulted in decreased or altered immune cell functions including expression of cell surface molecules, cytokine secretion, and proliferation in response to mitogen

stimulation. However, it should be noted that the *in vivo* cells of the immune system exist as part of a network influenced by other cell types. Therefore, the purification and isolation of the particular cell types to be studied often disrupts or alters such interactions, and interpretation of these types of results, including trying to determine their clinical relevance, can be difficult.

The effect of EPA and DHA intake on cell surface marker expression was examined when healthy volunteers supplemented their diets with 1.56 g EPA and DHA per day for 3 weeks (Hughes et al. 1996). There was a decreased level of major histocompatibility complex class II (MHC class II) expression on the surface of peripheral blood monocytes. The expression of MHC molecules on cell surfaces is required for antigen presentation during the initiation of the immune response. Although caution should be exercised in interpreting the results of this study, which involved a small number of participants (6), the findings support the possibility that fish oil may be beneficial in the treatment of autoimmune disorders, which are associated with the abnormally elevated expression of both MHC class II molecules.

Several studies have found changes in macrophage and monocyte-derived cytokine production (Endres et al. 1989; Meydani et al. 1991, 1993; Cooper et al. 1993) following fish oil consumption. Peripheral blood monocytes from healthy adults consuming 1.23 to 6 g of fish oil per day for 6 to 24 weeks produced less TNF-α and IL-1 than controls. Similarly, 4 weeks of supplementation of healthy volunteers with linseed or fish oil (2.7 g EPA and DHA per day) demonstrated slightly lower *ex vivo* production of TNF-αand IL-1, with the effect of fish oil being more pronounced (Caughey et al. 1996). In another study, healthy males supplemented their diet with 6 g DHA per day for 90 days (Kelley et al. 1999). Here, DHA significantly decreased IL-1, TNF-α, and PGE_2 production by stimulated peripheral blood mononuclear cells. NK cell activity in the DHA group was also significantly decreased compared to controls. Healthy males receiving DHA capsules only (6 g/day) did not demonstrate a decrease in NK cell activity (Thies et al. 2001a), although in the same study, a group that consumed fish oil containing both EPA and DHA (9 g/day) did show a significant decrease in NK activity after 12 weeks.

In addition, it has been demonstrated that fish oil diets alter lymphocyte-derived cytokine production as well. For example, supplementation of the diet of healthy volunteers with 1.23 or 2.4 g/day of EPA and DHA for 12 or 24 weeks lowered *ex vivo* IL-2 production (Meydani et al. 1991). Also, lymphocytes isolated from volunteers who supplemented their diet with 18 g of marine lipids (4.6 g EPA and DHA) per day for 6 weeks exhibited a significant decrease in IL-2 production (Endres et al. 1993). This was paralleled by a marked decrease in mitogen-induced proliferation, a functional parameter closely associated with IL-2 production. Interestingly, maximal suppression of IL-2 production was observed as late as 10 weeks after cessation of the fish oil supplementation. This is consistent with a previous study by Endres et al. (1989) that demonstrated significant decreases in TNF-α and IL-1 production as late as 10 weeks after supplementation with 4.55 g of EPA and DHA per day was discontinued. This phenomenon may be a result of re-utilization of n-3 fatty acids from a slow-turnover compartment of fatty acids.

Decreased lymphocyte proliferation in response to antigenic stimulation following fish oil consumption has been reported by several investigators (Meydani 1991; Meydani and Dinarello 1993; Kremer et al. 1987; Santoli and Zurier 1989; Kelly et al. 1991). However, Payan and

Goetzl (1983) found an increase in lymphocyte proliferation in asthma patients and Kremer et al. (1987) observed an increase in lymphocyte proliferation in patients with rheumatoid arthritis after fish oil consumption. The conflicting observations might be due to differences in health status, length of dietary treatment, drug use, patient age, and reagents used for the *in vitro* studies. Several immune parameters were examined in volunteers who supplemented their diets with daily doses of 2.4 g of EPA for 12 weeks (Virella et al. 1991). No consistent changes were observed in neutrophil function tests, mitogen-stimulated lymphocyte proliferation, and immunoglobulin and antibody synthesis. Production of IL-2 from lymphocytes following stimulation appeared to be decreased, although this effect was not significant.

Some studies have focused on the effect of fish oils on neutrophil function. Neutrophils are essential to host defenses against pathogens, although neutrophils often contribute to the pathology of several inflammatory disorders, in large part through the production of superoxide radicals. Healy et al. (2000) examined the effect of fish oil supplementation (up to 4 g of EPA and DHA per day) on neutrophil function in healthy volunteers after 12 weeks. Neutrophil fatty acid composition changed, but there was no difference in superoxide generation or chemotaxis compared to controls. Neutrophil functions have only been reported to be attenuated when the combined intakes of EPA and DHA were well above 5 g/day (Luostarinen et al. 1996; Sperling et al. 1993).

Although there are no human data describing a relationship between fish oil intake and decreased host defenses, a study in mice identified an effect of dietary fish oil on bacterial clearance (Fritsche et al. 1997). Female C3H mice were given special diets supplemented with menhaden fish oil (16% EPA and 12% DHA), lard or soybean oil. After 4 weeks of consuming the experimental diet, the mice were challenged with a sublethal dose of *Listeria monocytogenes*, an intracellular bacterium that has been used extensively as a model with which to study host resistance. The mice fed the fish oil demonstrated decreased bacterial clearance 4 days post-infection as compared to controls. These results are in contrast with those reported by Rubin et al. (1989). This study also examined the effect of dietary fish oil on the response to *Listeria* and failed to show an effect. This may be due to the strains of mice that were used for the different studies. Rubin et al. used NZB autoimmune-disease prone mice, which may be more resistant to *Listeria* than the C3H strain. This discrepancy underscores the uncertainty associated with establishing a relationship between a certain chemical and alterations in host resistance, even when using animal models.

One issue that was not raised by FDA is the potential for lactating women consuming fish oil supplements to exhibit an adverse effect on the immune components in their milk, with possible impact on the immune status of the nursing infant. Hawkes and her colleagues (2001, 2002) explored the question of whether dietary supplementation of lactating women with EPA and DHA can modulate the concentration of cytokines in the aqueous phase of human milk and the production of cytokines by human milk cells. In addition to a placebo group, they administered tuna oil containing either 370 mg EPA+DHA (70 mg EPA, 300 mg DHA) or 740 mg EPA+DHA (140 mg EPA, 600 mg DHA) daily to healthy post-partum lactating women for 4 weeks. While this supplementation did increase the n-3 PUFA concentrations in relevant tissues, it did not perturb the cytokine concentrations (IL-1β, IL-6, TNF-α, transforming growth factor-β1 (TGF-β1), and TGF-β2) in the milk.

It is difficult to assign clinical relevance to discrete alterations within the immune system. The cytokine network of the immune system is varied and redundant, e.g., several cytokines have the same effect on the same cell population. Therefore, one is unable to conclude that decreased cytokine production is an indicator of overall decreased host resistance following dietary administration of EPA and DHA. Similarly, *ex vivo* lymphocyte proliferation in response to mitogen is not reflective of *in vivo* conditions. In the host, lymphocyte proliferation following antigenic challenge is a multi-stage process that involves many cell types, and it is difficult to interpret these observations without considering overall host resistance to disease and infection. It should be noted, however, that the authors of the previously described studies in which test subjects consumed EPA- and DHA-containing fish oil did not describe any increased incidence of disease or infection. In addition, it is unclear from evidence generated in the animal studies described above whether or not EPA and DHA disrupt host resistance against pathogens. Furthermore, no human studies exist that support the hypothesis that consumption of fish oil causes increased susceptibility to infection and disease.

The results of some clinical studies have suggested a beneficial effect of fish oil (EPA and DHA) consumption on diseases or conditions associated with inflammation, but suppression or alteration of certain immune parameters could be significant, especially in those individuals with compromised immune systems. However, the EPA and DHA intakes at which significant alterations of immune function were observed were relatively high (4 to 9.75 g/person/day). Therefore, it is reasonable to suggest that consumption of fish oil resulting in EPA and DHA intakes of up to 3 g/day would not be expected to compromise overall host defenses, and thus would be protective of any potential immunosuppressive effects of EPA and DHA.

This conclusion is supported by the recent prepublication report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Macronutrients (FNB-IOM 2002). After reviewing the available literature regarding the impact of consumption of EPA and DHA on immune function, bleeding and increased risk of hemorrhagic stroke, and oxidative damage, the Committee concluded (pp. 8–57):

> While there is evidence to suggest that high intakes of n-3 polyunsaturated fatty acids, particularly EPA and DHA, may impair immune response and result in excessively prolonged bleeding times, it is not possible to establish a UL. Studies on immune function were done *in vitro* and it is difficult, if not impossible, to know how well these artificial conditions simulate human immune cell response *in vivo*. Data on EPA and DHA intakes and bleeding times are mixed and dose-response effect was not observed.

IV. SAFETY ASSESSMENT/GRAS DETERMINATION

A. Introduction

This chapter presents an assessment that demonstrates that 18/12 TG, in both oil and powder form, is safe, and is also GRAS under the FDCA for direct addition to foods as a nutrient supplement at specified use levels in a variety of foods, as listed in Table 2. This safety assessment and GRAS determination entails two steps. In step one, the safety of 18/12 TG under its intended conditions of use is demonstrated. Safety is established by showing that 18/12 TG is substantially equivalent to edible fish oils and fish oils already regarded as GRAS for addition to foods and comparing the EDIs of EPA+DHA and all impurities from 18/12 TG under its intended conditions of use with the ADIs for EPA+DHA and for all impurities. A substance directly added to food is considered safe for its intended use if the EDI of the substance under its intended conditions of use is less than or approximates its ADI (FDA 1993a). In the second step, 18/12 TG is determined to be GRAS by demonstrating that the safety of this substance under its intended conditions of use is generally recognized among qualified scientific experts.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FDCA is set forth under 21 CFR 170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under 21 CFR 170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under 21 CFR 170.30(c). This GRAS determination employs scientific procedures established under 21 CFR 170.30(b).

A key concept is that of substantial equivalence. In its proposed rule establishing the GRAS notification process (FDA 1997a), FDA cited the recommendations of a 1996 joint consultation by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). This consultation (FAO/WHO 1996) recommended that, "if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e. the food or food component can be concluded to be as safe as the conventional food or food component). Account should be taken of any processing that the food or food component may undergo as well as the intended use and the intake by the population."

In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether 18/12 TG, employed as a nutrient supplement, is safe and is also GRAS for the uses and at the use levels shown in Table 2. Once 18/12 TG, both oil and powder

(microcapsules), is determined to be GRAS for its intended uses, it is permitted to be used for those purposes because it is not (by definition) a food additive, and therefore does not require promulgation of a food additive regulation under 21 CFR prior to being lawfully marketed and sold in the U.S.

B. Safety of 18/12 TG Oil and Microcapsules

A scientific procedures GRAS determination requires first that information about the substance establish that the intended use of the substance is safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR 170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

1) The probable consumption of the substance and of any substance formed in or on food because of its use (i.e., the EDI);

2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet; and

3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

After consideration of these factors, an ADI and an EDI are typically derived for the substance. The ADI represents the maximum amount of the substance that has been shown to be safe for consumption by humans on a daily basis for a lifetime. An EDI for the substance is derived based on the probable human consumption of the substance and of any substance formed in or on food because of its use. Finally, the EDI for a substance is compared against its ADI. As long as the EDI is less than or approximates the ADI, the substance can be considered safe for its intended use (FDA 1993a).

As was noted earlier, 18/12 TG is substantially equivalent to other edible fish oils and also fish oils, such as menhaden oil and small planktivorous pelagic fish body oil (SPPFBO), that are already GRAS for addition to foods (FDA 1997b, FDA 2002c). The primary distinguishing characteristic of fish oils as compared with other oils used in foods is their higher content of the omega-3 fatty acids EPA and DHA. ONC's 18/12 TG contains, on average, about 30% EPA+DHA; SPPFBO also contains about 30% EPA+DHA while menhaden oil contains about 20%. The ratio of EPA:DHA in the three oils is similar: the ratio in 18/12 TG and in SPPFBO is about 1.5:1 and the ratio in menhaden oil is about 1.7:1. Since 18/12 TG is substantially equivalent to edible fish oils as well as these GRAS oils, it can be concluded to be as safe as they are, subject to considerations of the intended use and the intake by the population and processing to remove impurities.

1. EDI of EPA and DHA

As indicated above, 21 CFR 170.3(i) requires that, in evaluating the safety of the proposed use of a new food additive, the probable consumption (i.e., the EDI) of the substance and of any

substance formed in or on food because of its use be considered, as well as the cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet. Thus, because a scientific procedures GRAS determination requires the same quantity and quality of evidence as is required to obtain approval of the substance as a new food additive, a scientific procedures GRAS determination must also consider the probable consumption and cumulative effect of the substance in the diet.

The estimated mean potential intake of EPA+DHA from 18/12 TG from all proposed use categories by users of one or more foods is 3 g/day. The estimated mean potential intake of 18/12 TG oil is 10 g/day and the estimated mean potential intake of 18/12 TG powder (microcapsules) is 18 g/day. These figures are based on an average EPA+DHA content of 30% in the oil and 17% in the powder, which is 55–60% oil.

Results of the estimates of exposure from all food categories indicate that more than 99% of the U.S. population consume one or more of the foods and beverages included in the list of proposed uses over a nonconsecutive two-day period. The calculated EDIs are likely considerable overestimates of exposure to 18/12 TG, as these estimates assume that all foods in the proposed use categories contain 18/12 TG at the maximum proposed use levels. Intake of EPA+DHA from 18/12 TG does not increase the potential intake over that already provided by menhaden oil, SPPFBO, and other GRAS fish oils because all fish-oil products are alternatives that are used under the same restrictions regarding EPA+DHA addition. The cumulative EDI of EPA and DHA from both naturally occurring sources (0.1 g/person) and the proposed uses of 18/12 TG oil or microcapsules (3 g/person) is, therefore, about 3.1 g/person.

2. ADI for EPA and DHA

The FDA has previously reviewed safety concerns regarding consumption of fish oil containing the two omega-3 fatty acids EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS (FDA 1997b). The primary safety concerns evaluated by the FDA associated with excessive intakes of EPA and DHA included increased bleeding times, reduced glycemic control among diabetics, and increased levels of LDL cholesterol among diabetics and hyperglycemics. Based on this review, the FDA concluded that a combined intake of EPA and DHA of up to 3 g/person/day would not result in any adverse health effects. As described in Chapter III, an evaluation was conducted of the more recently published scientific literature to determine if there is any new information pertaining to the FDA's safety concerns that would contradict what was concluded and recommended by FDA in the 1997 review of EPA and DHA intake from fish oil. In addition, as was also described in Chapter III, because of a more recent concern raised by FDA regarding potential immunosuppressive effects of EPA and DHA, a review was conducted of the published literature relevant to the effects of EPA and DHA on the immune system. These reviews focused on studies of fish oils and of EPA and DHA in combination, rather than on the distinct metabolic effects of EPA and DHA ingested independently.

Based on the review presented in Chapter III, the following conclusions were reached regarding a safe level of intake of EPA and DHA from consumption of fish oil:

- The totality of the evidence found in the published scientific literature demonstrates that, when consumption of EPA and DHA is limited to 3 g/person/day or less, there is no significant risk for increased bleeding time above the reported normal range.

- Evaluation of more recent data on the effects of fish oil containing EPA and DHA on glycemic control indicates that there is no new evidence to suggest that EPA and DHA intakes of less than 3 g/day will adversely affect glycemic control in diabetics.

- An examination of the recent literature regarding the effect of EPA- and DHA-containing fish oil on LDL is consistent with the conclusion reached by the FDA that daily consumption of fish oils resulting in intakes of EPA and DHA less than 3 g/day does not adversely influence LDL levels.

- Consumption of fish oil resulting in EPA and DHA intakes of up to 3 g/day would not be expected to compromise overall host defenses, and thus would be protective of any potential immunosuppressive effects of EPA and DHA.

Therefore, the more recent literature is consistent with FDA's determination that the ADI for EPA and DHA combined from the consumption of fish oil is 3 g/person/day, and that this ADI can be used to evaluate the safety of 18/12 TG for direct addition to food. The evidence is less clear with respect to the independent effects of EPA and DHA, but there is nothing in the literature that would indicate a potential hazard due to intakes of either fatty acid in the range of 1–2 g/person/day with an intake of the two combined not exceeding 3 g/person/day.

3. Establishing the Safety of 18/12 TG Oil and Microcapsules

As a result of the proposed uses and use level of 18/12 TG oil and microcapsules, the EDI of EPA+DHA is about 3 g/person/day. To this EDI of EPA and DHA from 18/12 TG, the potential contribution of EPA and DHA from naturally occurring sources must be added. EPA and DHA from these natural sources could contribute as much as 0.1 g/person/day, yielding a cumulative EDI of 3.1 g per person.

The cumulative EDI of EPA and DHA of 3.1 g/person/day, due to the addition of 18/12 TG oil or microcapsules to various foods at the proposed use levels and intake from naturally occurring sources, approximates the ADI for EPA and DHA of 3 g/day established by the FDA and confirmed by an assessment of research published since the FDA review. Furthermore, the EDIs of all impurities in 18/12 TG oil are less than their respective ADIs. Thus, the proposed uses and use levels of 18/12 TG can be considered safe.

C. General Recognition of the Safety of 18/12 TG Oil and Microcapsules

The proposed uses and use level of 18/12 TG have been determined to be safe through scientific procedures set forth under 21 CFR 170.30(b). This safety was established by demonstrating that 18/12 TG is substantially equivalent to other fish oils already GRAS for addition to foods, followed by estimating potential human exposure to EPA and DHA from the intended uses of 18/12 TG. Next, the FDA's determination that an intake of EPA and DHA of up to 3 g/person/day from consumption of menhaden oil is safe was employed to establish an ADI for EPA+DHA of 3 g/person/day. The FDA scientists who participated in this determination are

considered to be experts qualified by scientific training and experience to evaluate safe levels of exposure to EPA and DHA. The published scientific literature on which the FDA's safe intake level was based was updated and reviewed to confirm that the ADI for EPA and DHA established by FDA is consistent with current information. Then, the probable human exposure, or EDI, for EPA and DHA, resulting from the proposed uses and use level of 18/12 TG in food, was compared to the ADI for EPA and DHA. Because the EDI approximates the ADI, and EDIs for no impurities exceed their respective ADIs, 18/12 TG can be considered safe for its intended uses at the specified use levels. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of 18/12 TG for direct addition to foods in either oil or powder form under the intended conditions of use has been made through the deliberations of Robert G. Ackman, Ph.D., Joseph F. Borzelleca, Ph.D., and Walter H. Glinsmann, M.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, and have concluded:

> *ONC's 18/12 TG is substantially equivalent to other fish oils that are already GRAS for addition to foods. No evidence exists in the available information on EPA and DHA that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when EPA and DHA are used at levels that are now current or that might reasonably be expected from the proposed uses of ONC's 18/12 TG oil or powder as a nutrient supplement.*

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, 18/12 TG is safe and is GRAS for the proposed uses described in Table 2. Because ONC's fish oil is GRAS for its proposed uses, it is excluded from the definition of a food additive, and thus may be lawfully marketed and sold for these uses in the U.S. without the promulgation of a food additive regulation under 21 CFR.

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End Submission

JHeimbach LLC

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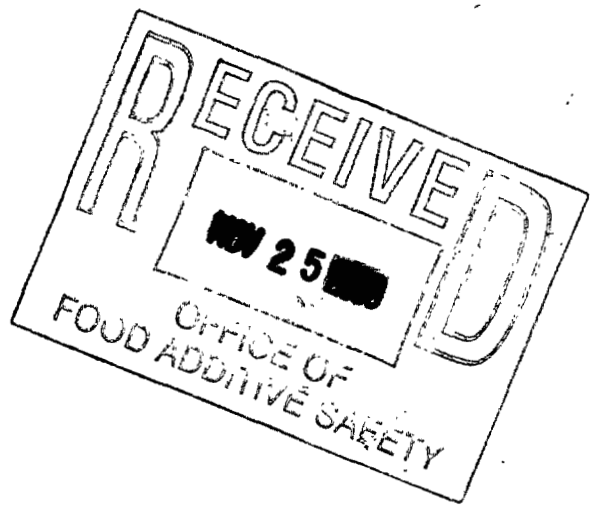


November 18, 2003

Linda S. Kahl, Ph.D.
Division of Biotechnology
and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

GRN 138

Dear Linda:

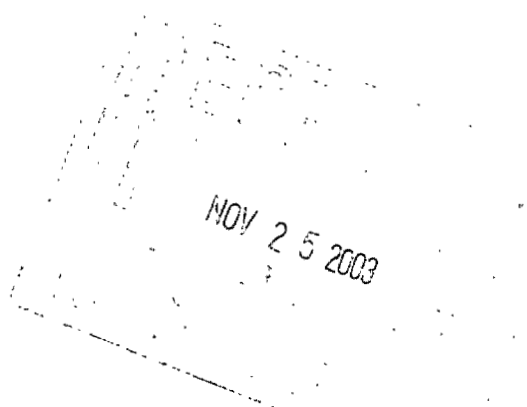
As discussed in our telephone call, the GRAS notices enclosed are intended as an amendment to the GRAS notice I sent on September 26. This amendment provides additional information requested by Carrie Hendrickson and her colleagues in a telephone conference yesterday.

As required, three copies are provided.

If you have any questions regarding this amendment, please feel free to contact me at 202-237-8406 or jim@jheimbach.com.

Sincerely,

James T. Heimbach, Ph.D., F.A.C.N.



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I. GRAS EXEMPTION CLAIM

Ocean Nutrition Canada, Ltd. (ONC), through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration the use of 18/12 TG described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because ONC has determined that such use is generally recognized as safe (GRAS).

11/17/03

James T. Heimbach, Ph.D., F.A.C.N. — Date
President, JHEIMBACH LLC
Agent for Ocean Nutrition Canada, Ltd.

A. Name and Address of Notifier

Ocean Nutrition Canada Ltd.
757 Bedford Highway
Bedford, Nova Scotia B4A 3Z7
Canada

Contact: Janet Shay
Telephone: (902) 457-5908
Facsimile: (902) 445-2220
E-mail: jshay@ocean-nutrition.com

B. Name of GRAS Substance

The common name of the substance that is the subject of this Generally Recognized As Safe (GRAS) notification is 18/12 TG derived from fish oil by Ocean Nutrition Canada Ltd. (ONC). The crude fish oil employed in the production of 18/12 TG is extracted from multiple edible marine fish species caught off the coast of Peru. These fish species include anchovy (95–99%), sardine (1–5%), jack mackerel, Pacific mackerel, and other occasional species. Approximately 30%, on average, of 18/12 TG is composed of the two omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), primarily in the form of triacylglycerides. The total content of EPA+DHA ranges from 23 to 36%, with EPA constituting between 10–21% and DHA 8–20% of the product.

The 18/12 TG is substantially similar to other edible fish oils, as shown by the comparison of fatty acid profiles shown in Table 1. It is also substantially similar to other fish oils that are already regarded as GRAS for addition to foods, including menhaden oil (21 CFR 184.1472) and small planktivorous pelagic fish body oil (SPPFBO, GRAS Notice GRN 102; FDA 2002c). This latter fish oil is derived primarily from sardine and anchovy, the same fish that are the principal sources of 18/12 TG. Menhaden oil contains only 20-22% EPA+DHA, while SPPFBO and

18/12 TG both contain approximately 30% EPA+DHA; however, the average ratios of EPA:DHA in the three oils are similar, 1.7:1, 1.5:1, and 1.5:1 respectively.

The 18/12 TG may be sold either in the form of free oil or in microencapsulated form where the 18/12 TG constitutes approximately 55–60%, by weight, of the powder. The microencapsulated form of the product is known as Microencapsulated Fish Oil or Microencapsulated Omega-3 Fish Oil.

C. Intended Use and Consumer Exposure

ONC intends to market its 18/12 TG, in both the oil and microencapsulated forms, for addition to several categories of foods as a nutrient supplement (21 CFR 170.3(o)(20)) to increase the dietary intake of the omega-3 fatty acids EPA and DHA. The food categories proposed for addition and the proposed addition levels are listed in Table 2. These are the same food categories as are specified in the GRAS regulation for menhaden oil (21 CFR 184.1472(a)(3)), and 18/12 TG thus serves as an alternative to menhaden oil as a source of EPA and DHA. The 18/12 TG oil is proposed for addition at 67% of the use levels proposed for menhaden oil (also shown in Table 2), reflecting the average 30% EPA+DHA composition of 18/12 TG compared with 20% in menhaden oil. The 18/12 TG microcapsules are proposed for use at 120% of the use levels proposed for menhaden oil, reflecting their average 17% EPA+DHA composition. Thus, the addition rates of EPA+DHA are the same for all three fish oil products, as shown in the last column of Table 2.

It is intended that 18/12 TG will be used as the sole added source of EPA and DHA in any given food category and is not to be combined or augmented with any other source of EPA or DHA in making a food product.

On February 26, 2002, FDA issued a proposed rule (67 FR 8744) that would amend 21 CFR 184.1472(a)(3) by reallocating the uses of menhaden oil in a different set of food categories, each with a specified maximum level of use. ONC intends that any changes to the permitted uses of menhaden oil specified in 21 CFR 184.1472(a)(3) would also apply to 18/12 TG. In other words, the levels of use of 18/12 TG oil would be 67% of whatever maximum levels of use are specified in 21 CFR 184.1472(a)(3) and the levels of use of 18/12 TG microcapsules would be 120% of whatever maximum levels of use are specified in 21 CFR 184.1472(a)(3); in both cases, the permitted categories of foods would be the same. These potential future levels of use are shown in Table 3.

As with the use of menhaden oil, the maximum levels of use of 18/12 TG oil and microcapsules are designed to assure that the combined daily intake of EPA and DHA will not exceed 3 g/person/day.

The estimated mean intake of EPA and DHA combined from the proposed uses of ONC's 18/12 TG listed in Table 2 (or in Table 3) by consumers age 2 years and older does not exceed 3 g/person/day. Cumulative intake of EPA and DHA from food sources by this population, including intakes from both the proposed uses of the 18/12 TG and naturally occurring sources of fish oil, is estimated to be less than 3.1 g/person/day.

D. Basis for GRAS Determination

ONC's GRAS determination for the proposed uses of its 18/12 TG oil and microcapsules listed in Table 2 is based on scientific procedures as described under 21 CFR 170.30(b).

ONC's 18/12 TG has been shown to be substantially equivalent to other edible fish oils (see Table 1), including fish oils that are already GRAS for addition to foods. The estimated intake of 18/12 TG from the intended uses specified in Table 2, in addition to intakes of EPA and DHA from natural fish oil sources, is safe and is also GRAS under the Federal Food, Drug, and Cosmetic Act (FDCA). To demonstrate that ONC's 18/12 TG is GRAS under its intended conditions of use, the safety of both whole product intake and EPA+DHA intake from consumption of ONC's 18/12 TG is established under its intended conditions of use, taking into account potential intake of EPA and DHA from natural sources in the diet. Then, this intake of the whole product and of EPA+DHA is determined to be GRAS by showing that the safety of these levels of intake is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, and is based on generally available and accepted information.

In affirming the GRAS status of menhaden oil (21 CFR 184.1472), FDA has previously derived an acceptable daily intake (ADI) for EPA+DHA of 3 g/person/day (FDA 1997b). The FDA scientists who participated in this ADI derivation are considered to be experts qualified by scientific training and experience to evaluate safe levels of exposure to EPA and DHA. Reviews of the scientific literature published since the date of the FDA GRAS affirmation confirm that the ADI for EPA+DHA established by the FDA is consistent with all available current information regarding the safety of consumption of EPA and DHA.

The safety of 18/12 TG intake under its intended conditions of use is evaluated through an estimate of the potential exposure to EPA and DHA from both current uses of fish oil and proposed uses of 18/12 TG, and then this cumulative estimated daily intake (EDI) is compared with the ADI established by the FDA for EPA and DHA of 3 g/person/day. If the EDI is less than or approximates the ADI, the proposed uses of 18/12 TG can be considered safe.

The cumulative EDI of EPA and DHA from consumption of 18/12 TG and from naturally occurring fish oil sources in the general population, excluding infants under the age of one year, is less than 3.1 g/person/day for the average consumer. This intake estimate reflects 100% market penetration of the proposed uses listed in Table 2 and thus is a considerable overestimate of likely consumption. This cumulative EDI of under 3.1 g/day of EPA and DHA in the general population approximates the ADI for EPA and DHA of 3 g/day established by the FDA. Therefore, 18/12 TG, in either oil or microencapsulated form, is safe under its intended conditions of use.

The publicly available data demonstrating the safety of the proposed uses of 18/12 TG oil and microcapsules was reviewed by a GRAS panel consisting of Robert G. Ackman, Ph.D., Joseph F. Borzelleca, Ph.D., and Walter H. Glinsmann, M.D. This panel evaluated the dietary exposure, source of the substance, method of manufacture, specifications, and contaminant levels, as well as information from recent published toxicological and human studies. The GRAS panel, which ONC regards as qualified by scientific training and experience to evaluate the safety of

substances added to food, concluded that 18/12 TG oil and microcapsules, meeting food grade specifications, are GRAS under their intended conditions of use.

Therefore, it is concluded, based on scientific procedures, that the intended use of ONC's 18/12 TG, as shown in Table 2, is safe and is also GRAS.

E. Availability of Information

Much of the information in this GRAS notification, particularly the review of the safety of EPA and DHA and the application of the TEF process to dioxin-like compounds, was originally prepared for ONC by ENVIRON International Corporation in 2001; it has since been expanded and updated. The data and information that serve as the basis for this GRAS notification will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHEIMBACH LLC, 4530 Broad Branch Road, NW, Washington, DC 20008, telephone: (202) 237-8406, e-mail jim@jheimbach.com.

Table 1. Fatty Acid Profiles of 18/12 TG And Typical Edible Fish Oils				
Fatty Acid	**Percent by Weight**			
	18/12 TG	**Herring**	**Salmon**	**Sardine**
14:0	7.42	7.19	3.28	6.52
16:0	17.05	11.70	9.84	16.65
18:0	3.51	0.82	4.24	3.89
16:1 undiff	8.46	9.64	4.82	7.51
18:1 undiff	12.60	11.96	16.98	14.75
20:1 undiff.	0.00	13.62	3.86	5.99
22:1 undiff	0.00	20.61	3.38	5.59
18:2 undiff.	1.47	1.15	1.54	2.01
18:3 undiff.	1.51	0.76	1.06	1.33
18:4 undiff.	3.05	2.30	2.80	3.02
20:4 undiff.	2.08	0.29	0.68	1.76
20:5 n-3	18.55	6.27	13.02	10.14
22:5 n-3	2.40	0.62	2.99	1.97
22:6 n-3	11.85	4.21	18.23	10.66

Sources of Data:
18/12 TG: Average of 5 lots produced by ONC
Herring, salmon, sardine oils: USDA Nutrient Database for Standard Reference, Release 16

Table 2. Maximum Levels of Use of Menhaden Oil, 18/12 TG Oil, and 18/12 TG Microcapsules and Resulting Addition Levels of EPA+DHA

Category of Food[1]	Maximum Level of Use			EPA+DHA Addition Level
	Menhaden Oil[2]	18/12 TG		
		Oil	Micro-capsules	
Cookies and crackers (1)	5.0%	3.3%	6.0%	1.0%
Breads and rolls (white and dark) (1)	1.0%	0.7%	1.2%	0.2%
Fruit pies and custard pies (1)	7.0%	4.7%	8.4%	1.4%
Cakes (1)	10.0%	6.7%	12.0%	2.0%
Cereals (4)	4.0%	2.7%	4.8%	0.8%
Fats and oils (12), not in infant formula	20.0%	13.4%	24.0%	4.0%
Yogurt (31)	4.0%	2.7%	4.8%	0.8%
Cheese products (5)	5.0%	3.3%	6.0%	1.0%
Frozen dairy products (20)	5.0%	3.3%	6.0%	1.0%
Meat products (29)	10.0%	6.7%	12.0%	2.0%
Egg products (11)	5.0%	3.3%	6.0%	1.0%
Fish products (13)	20.0%	13.4%	24.0%	4.0%
Condiments (8)	5.0%	3.3%	6.0%	1.0%
Soup mixes (40)	3.0%	2.0%	3.6%	0.6%
Snack foods (37)	5.0%	3.3%	6.0%	1.0%
Nut products (32)	5.0%	3.3%	6.0%	1.0%
Gravies and sauces (24)	5.0%	3.3%	6.0%	1.0%

1 The number in parenthesis following each food category is the paragraph listing of that food category in 21 CFR 170.3(n).

2 Food categories and maximum use levels as specified in 21 CFR 184.1472(a)(3).

Table 3. Maximum Future Levels of Use of Menhaden Oil, 18/12 TG Oil, and 18/12 TG Microcapsules and Resulting Addition Levels of EPA+DHA Under the Proposed Rule

Category of Food*	Maximum Level of Use			EPA+DHA Addition Level
	Menhaden Oil	18/12 TG		
		Oil	Micro-capsules	
Baked goods and baking mixes (1)	5.0%	3.3%	6.0%	1.0%
Cereals (4)	4.0%	2.7%	4.8%	0.8%
Cheese products (5)	5.0%	3.3%	6.0%	1.0%
Condiments (8)	5.0%	3.3%	6.0%	1.0%
Egg products (11)	5.0%	3.3%	6.0%	1.0%
Fats and oils (12), not in infant formula	12.0%	8.0%	14.4%	2.4%
Fish products (13)	5.0%	3.3%	6.0%	1.0%
Frozen dairy desserts (20)	5.0%	3.3%	6.0%	1.0%
Gravies and sauces (24)	5.0%	3.3%	6.0%	1.0%
Meat products (29)	5.0%	3.3%	6.0%	1.0%
Milk products (31)	5.0%	3.3%	6.0%	1.0%
Nut products (32)	5.0%	3.3%	6.0%	1.0%
Snack foods (37)	5.0%	3.3%	6.0%	1.0%
Soup mixes (40)	3.0%	2.0%	3.6%	0.6%
Nonalcoholic beverages (3)	0.5%	0.3%	0.6%	0.1%
Chewing gum (6)	3.0%	2.0%	3.6%	0.6%
Confections and frostings (9)	5.0%	3.3%	6.0%	1.0%
Dairy product analogs (10)	5.0%	3.3%	6.0%	1.0%
Gelatins and puddings (22)	1.0%	0.7%	1.2%	0.2%
Pastas (23)	2.0%	1.3%	2.4%	0.4%
Hard candy (25)	10.0%	6.7%	12.0%	2.0%
Jams and jellies (28)	7.0%	4.7%	8.4%	1.4%
Plant protein products (33)	5.0%	3.3%	6.0%	1.0%
Poultry products (34)	3.0%	2.0%	3.6%	0.6%
Processed fruit juices (35)	1.0%	0.7%	1.2%	0.2%
Processed vegetable juices (36)	1.0%	0.7%	1.2%	0.2%
Soft candy (38)	4.0%	2.7%	4.8%	0.8%
White granulated sugar (41)	4.0%	2.7%	4.8%	0.8%
Sugar substitutes (42)	10.0%	6.7%	12.0%	2.0%
Sweet sauces, toppings, and syrups (43)	5.0%	3.3%	6.0%	1.0%

* The number in parenthesis following each food category is the listing in 21 CFR 170.3(n).

II. IDENTITY OF THE SUBSTANCE

A. Chemical Name

Because the 18/12 TG that is the subject of this GRAS notification is a mixture of fatty acids, no single chemical name exists for this substance. The primary components are triacylglycerides that include EPA and DHA. The 18/12 TG includes ~30% of these two fatty acids combined (with a permissible range of 23 to 36%); EPA constitutes between 10% and 21% of the oil and DHA constitutes between 8 and 20%.

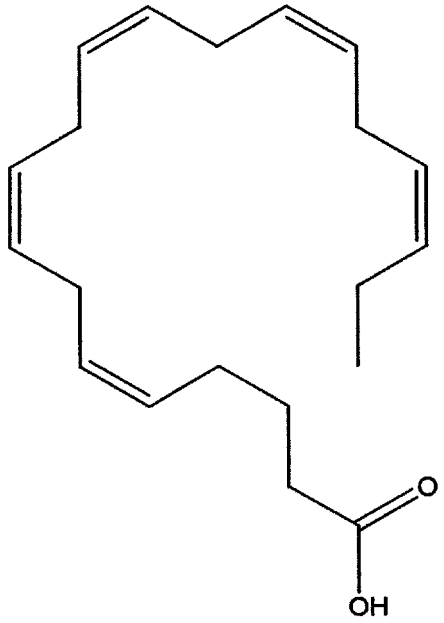
B. CAS Registry Number

Because the 18/12 TG that is the subject of this GRAS notification is a mixture of fatty acids, no Chemical Abstracts Service (CAS) Registry Number exists for this substance. The CAS Registry Numbers for EPA and DHA, the primary components of this product, are 10417-94-4 and 25167-62-8, respectively.

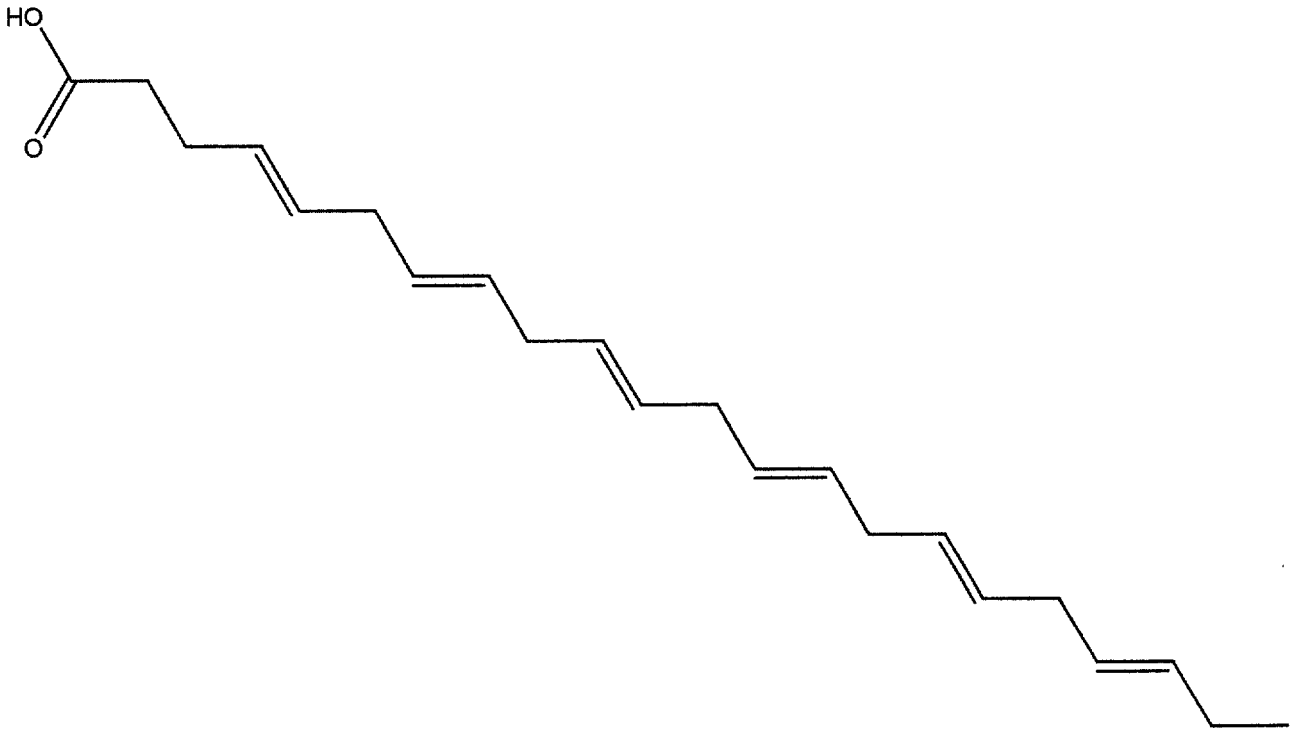
D. Molecular and Structural Formulas

Because the 18/12 TG that is the subject of this GRAS determination is a mixture of fatty acids, no single molecular or structural formula exists for this substance. The molecular formula for EPA is $C_{20}H_{30}O_2$, while the molecular formula for DHA is $C_{22}H_{32}O_2$. The structural formulas for these two major components of the 18/12 TG are shown in Figure 1.

Figure 1. Structural Formulas for EPA and DHA



Eicosapentaenoic Acid (EPA)



Docosahexaenoic Acid (DHA)

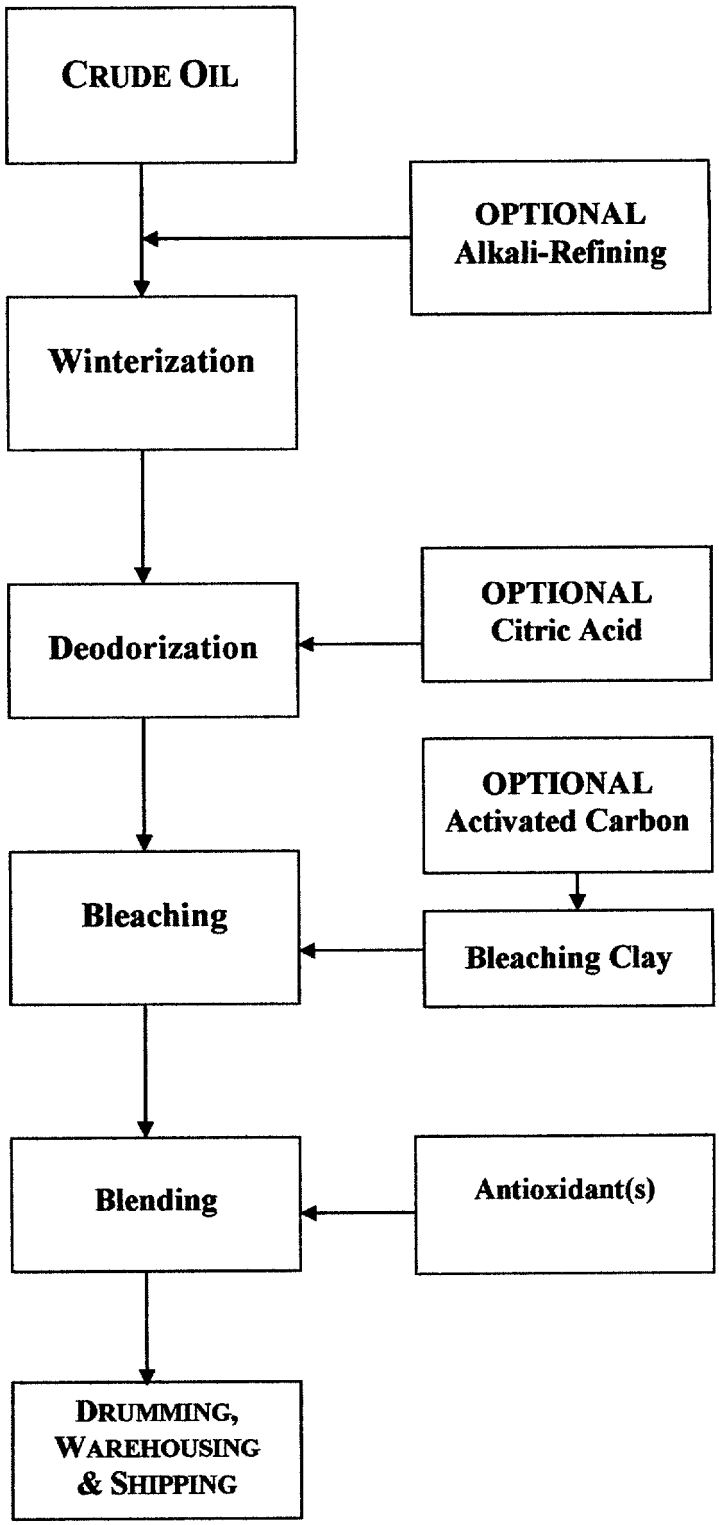
E. Source Material and Production/Purification Process for 18/12 TG

Producing 18/12 TG suitable for direct addition to foods involves processing and purifying the raw crude fish oil received from ONC's supplier. This production and purification process involves four or five basic steps:

1) (OPTIONAL) Alkali-refining
2) Winterization
3) Deodorization, either steam or wiped-film-evaporator
4) Bleaching with bleaching clay, with activated carbon optionally added
5) Blending with the addition of mixed natural tocopherols or other antioxidants

A generalized schematic of the production/purification process is shown in Figure 2. The processing steps do not necessarily occur in the order shown and, if needed, some of them (such as winterization) may be repeated. Each of these steps is discussed in greater detail below after characterization of the raw material employed in this process, crude bulk fish oil.

Figure 2. Generalized Schematic of the Production Process for 18/12 TG



1. Raw Material Specifications

The crude bulk fish oil that is the primary raw material for the production/purification process is a mixture of fatty acids (as triacylglycerides) extracted from multiple edible marine fish species caught off the coast of Peru. These fish species include anchovy (95–99%), sardine (1–5%), jack mackerel, Pacific mackerel, and other occasional species. ONC has established the following raw material specifications for the crude bulk fish oil it purchases from its supplier:

- Appearance: An amber-colored, free-flowing oil
- Odor: Characteristic of fish oil (i.e., natural fish smell)
- EPA content: 10% to 21%
- DHA content: 8% to 20%
- EPA+DHA content: 23% to 36%
- Gadoleic acid content: Not more than (NMT) 2%
- Cetoleic and erucic acid content: NMT 2%
- Acid value: NMT 10 mg KOH/g
- Peroxide value: NMT 20 meq/kg
- Anisidine value: NMT 25
- Totox number: NMT 65 (anisidine value + [2 X peroxide value])
- Arsenic: NMT 10 ppm
- Lead: NMT 0.1 ppm
- Cadmium: NMT 0.1 ppm
- Mercury: NMT 0.01 ppm

2. Alkali Refining (Optional)

In this optional process, commonly referred to as neutralization, an alkali, either NaOH or KOH, is added to the crude or winterized oil in the presence of heat to react with the free fatty acids to form soaps, which are then centrifuged out. Three water washes assure that the neutralization is complete. Both NaOH and KOH are affirmed GRAS for this process under 21 CFR 184.1763 and 184.1631, respectively, limited only by current good manufacturing practices (cGMP). This step may be performed by the supplier of the crude bulk oil prior to the product reaching the processing facilities of ONC.

3. Winterization

During winterization, also referred to as cold filtration, the oil is cooled to 0°C to crystallize the higher melting triacylglycerides; it is then passed through a filter press, while maintaining a temperature of not more than 5°C.

4. Deodorization

Deodorization may be accomplished by either of two methods, a continuous-flow process or a batch process. In the continuous flow method, crude bulk fish oil (which may have been alkali refined and/or winterized) is passed under high vacuum and at an elevated temperature through a

wiped film evaporator (WFE) at a rate of up to 500 kg/hour. In the batch process, steam deodorization, the temperature of the oil is raised to 195°C under full vacuum, after which steam is injected into the oil to remove the undesirable components.

In both processing methods, the high vacuum and elevated temperature remove polycyclic aromatic hydrocarbons (PAHs), PCBs, dioxins, furans, free fatty acids, and some sterols. The distillate is considered waste, while the residue—deodorized oil—enters the next step in the process.

5. Bleaching

Various methods of processing oils can cause darkening of the oil. Therefore, so-called "bleaching" is commonly employed to lighten the color of fish oils and other oils. Oils can be bleached by both physical and chemical means, but the most commonly used method for marine oils is a physical process—adsorption of the coloring matter using an activated earth or clay. Another physical method for bleaching, less commonly practiced, is heating the oil under vacuum (Bailey 1952). In the final step of ONC's refining process, both physical methods of bleaching described above are employed.

6. Blending and Addition of Antioxidants

As the last step in processing, the winterized, deodorized, bleached oil is blended with mixed tocopherols or other appropriate antioxidants and agitated to assure proper mixing.

F. Product Characteristics of 18/12 TG Oil

1. Specifications for the Food-Grade Material

ONC has developed specifications for 18/12 TG oil to demonstrate that it is food grade. These specifications are listed below, along with the reference to the ONC standard operating procedure (SOP) for determining compliance with each specification (except for total PCBs and metals, which are analyzed by a third-party contract laboratory using accepted validated methods for these contaminants).

• Description:	Food-grade 18/12 TG is a mixture of fatty acids (as triacylglycerides) of fish oil origin, extracted from multiple edible marine fish species caught off the coast of Peru, including anchovy (95–99%), sardine (1–5%), jack mackerel, Pacific mackerel, and other occasional species, and refined with standard methods.
• Appearance:	A clear, light yellow, free-flowing oil without sediment at room temperature (21°C) that is free from foreign matter and immiscible in water (SOP 85/05/938 and SOP 85/05/949)
• Odor and taste:	Characteristic of fish oil with no trace of rancidity or other abnormalities when tested organoleptically (SOP 85/05/906)
• EPA content:	Not less than (NLT) 10% and not more than (NMT) 21% (SOP 85/05/955), or NLT 90 mg/g and NMT 190 mg/g (SOP 85/05/917)
• DHA content:	NLT 8% and NMT 20% (SOP 85/05/955), or NLT 70 mg/g and NMT 190 mg/g (SOP 85/05/917)
• EPA + DHA:	NLT 23% and NMT 36%
• Gardner Color:	NMT 6 (SOP 85/05/927)
• Acid value:	NMT 2.0 mg KOH/g (SOP 85/05/904)
• Free fatty acid:	NMT 1.0% as oleic acid (SOP 85/05/904)
• Peroxide value:	NMT 10 meq/kg (SOP 85/05/907)
• *p*-Anisidine value:	NMT 25 (SOP 85/05/922)
• Totox number:	NMT 45 (anisidine value + [2 X peroxide value])
• Moisture content:	NMT 0.1% (SOP 85/05/918)
• Total PCBs:	NMT 2.0 ppm (USEPA Method 1668A)
• Arsenic (As):	NMT 3.5 ppm
• Lead (Pb):	NMT 0.1 ppm
• Cadmium (Cd):	NMT 0.1 ppm
• Mercury (Hg):	NMT 0.01 ppm

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2. Batch Analysis Results

To demonstrate conformance with the proposed specifications listed above, ONC analyzed several batches or lots of its final (i.e., post-bleached) 18/12 TG oil. The results of these

analyses are displayed in Table 4. These results show that all four lots of the oil are in full compliance with the established specifications, and thus the production process is under control.

Table 4. Batch Analysis Results for Four Lots of 18/12 TG

Parameter	Specification	Lot Number			
		2837	3813	4050	4098
Appearance	Clear yellow oil	Clear bright yellow oil	Clear yellow oil	Clear orange oil	Clear yellow oil
Odor	Characteristic of fish oil	Fishy	Slightly linseed	Slightly linseed	Slightly linseed
Taste	Characteristic of fish oil	Fishy	Slightly linseed	Slightly linseed	Slightly linseed
EPA (area %)	10–21	18.9	18.7	18	19
EPA (mg/g)	90–190	166.8	163.96	160.24	168.2
DHA (area %)	8–20	11.1	12.0	16.5	13.1
DHA (mg/g)	70–190	103.14	113.78	153.18	119.44
EPA plus DHA (%)	23–36	30.0	30.7	34.5	32.1
Acid value (mg KOH/g)	NMT 2.0	0.37	0.65	0.56	0.28
Peroxide value (meq/kg)	NMT 10	0.3	1.49	0.9	0.6
p-Anisidine value	NMT 25	6.87	8.31	5.62	8.04
Totox number	NMT 45	7.47	11.29	7.42	9.24
Gardner color	NMT 6	5	6	6	5
Free fatty acid (%)	NMT 1.0	0.19	0.33	0.28	0.14
Moisture content (%)	NMT 0.1	0.05	0.02	0.03	0.02
Density	No specification	Not tested	0.93142	0.93010	0.92876
Cold Test	Clear at 0°C for 24 hours	Not tested	Pass	Pass	Pass
Total PCBs (ppm)	NMT 2.0	0.006104	0.010754	0.003151	0.000681
Arsenic (ppm)	NMT 3.5	<0.1	<0.1	<0.1	<0.1
Lead (ppm)	NMT 0.1	<0.1	<0.1	<0.1	<0.1
Cadmium (ppm)	NMT 0.1	<0.02	<0.02	<0.02	<0.02
Mercury (ppm)	NMT 0.01	<0.01	<0.01	<0.01	<0.01

3. Contaminants

Although specifications were not established for these compounds, analyses were conducted for the following contaminants in conjunction with the batch analyses described above:

- Pesticides: Extensive analyses for pesticide residues, using methods with very low limits of detection (LODs), detected no pesticide residues in any of the four lots tested. Results for the four tested lots are shown in Table 5.

- Dioxins: The octa- congener was detected in all three lots of 18/12 TG tested, while the hepta- congener and the three hexa- congeners were detected in one lot each. Results for all three lots are shown in Table 6.

- Furans: Furan congeners were detected in all three lots of 18/12 TG tested (Table 7). One penta- congener was detected in two lots and one hepta- congener was detected in one lot.

- PCBs A number of PCBs were detected, all except three congeners in one lot of oil at levels of less than 1 ppb. Table 8 shows the results for the four lots tested; the highest total PCB content was 0.01 ppm in Lot 3813, about 1/20 of the specification for food-grade material.

- PAHs: Results for all four lots tested are shown in Table 9; PAHs were detected in all lots tested, none at levels exceeding 63 ppb.

Table 5. Pesticide Analysis Results for Four Lots of 18/12 TG

Pesticide	Concentration			
	Lot 2837	Lot 3813	Lot 4050	Lot 4098
Organochlorine (all ND)				
HCB	<0.001 ppm	<0.001 ppm	<0.001 ppm	<0.001 ppm
Lindane	<0.001 ppm	<0.001 ppm	<0.001 ppm	<0.001 ppm
Alpha HCH	<0.001 ppm	<0.001 ppm	<0.001 ppm	<0.001 ppm
Beta HCH	<0.001 ppm	<0.001 ppm	<0.001 ppm	<0.001 ppm
Delta HCH	<0.001 ppm	<0.001 ppm	<0.001 ppm	<0.001 ppm
Heptachlor	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
Aldrin	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
Chlordane	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
pp DDE	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
op DDE	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
pp DDD	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
op DDD	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
pp DDT	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
op DDT	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
Heptachlor epoxyde	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
Dieldrin	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Endrin	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Methoxychlor	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Toxaphene	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
PCB	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Endosulfan	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
Nitrogen (All ND)				
Dichlorbenil	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Diclofop-methyl	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Capcafol	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Captan	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Procymidone	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Vinclozolin	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Propoxur	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Amitraz	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm

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Table 5. Pesticide Analysis Results for Four Lots of 18/12 TG

Pesticide	Concentration			
	Lot 2837	Lot 3813	Lot 4050	Lot 4098
Phosphor (All ND)				
Azinphos-methyl	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Bromofos-ethyl	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Chlorfenvinfos	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Chlorpyrifos	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Diazinon	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Dichlorvos	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Disulfoton	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Ethion	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Fenitrothion	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Fensulfothion	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Fenthion	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
Malathion	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Methidathion	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Mevinphos	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Naled	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Parathion-ethyl	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Parathion-methyl	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm
Fosfamidon	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Pirimiphos-ethyl	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Pirimiphos-methyl	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Sulfotep	<0.002 ppm	<0.002 ppm	<0.002 ppm	<0.002 ppm
Trichlorofon	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm

Table 6. Dioxin Analysis Results for Three Lots of 18/12 TG

Dioxin	Concentration		
	Lot 3813	Lot 4050	Lot 4098
2,3,7,8-TCDD	ND (0.03 ppt)	ND (0.03 ppt)	ND (0.07 ppt)
1,2,3,7,8-PeCDD	ND (0.03 ppt)	ND (0.03 ppt)	ND (0.05 ppt)
1,2,3,4,7,8-HxCDD	ND (0.05 ppt)	ND (0.04 ppt)	0.05 ppt
1,2,3,6,7,8-HxCDD	ND (0.03 ppt)	ND (0.04 ppt)	0.09 ppt
1,2,3,7,8,9-HxCDD	ND (0.03 ppt)	ND (0.03 ppt)	0.07 ppt
1,2,3,4,6,7,8-HpCDD	ND (0.13 ppt)	0.27 ppt	ND (0.83 ppt)
OCDD	0.91 ppt	2.28 ppt	3.02 ppt

Table 7. Furan Analysis Results for Three Lots of 18/12 TG

Furan	Concentration		
	Lot 3813	Lot 4050	Lot 4098
2,3,7,8-TCDF	ND (0.21)	ND (0.15 ppt)	ND (0.11 ppt)
1,2,3,7,8-PeCDF	ND (0.03 ppt)	ND (0.03 ppt)	ND (0.06 ppt)
2,3,4,7,8-PeCDF	0.04	ND (0.03 ppt)	0.03 ppt
1,2,3,4,7,8-HxCDF	ND (0.03 ppt)	ND (0.03 ppt)	ND (0.11 ppt)
1,2,3,6,7,8-HxCDF	ND (0.03 ppt)	ND (0.03 ppt)	ND (0.04 ppt)
2,3,4,6,7,8-HxCDF	ND (0.04 ppt)	ND (0.04 ppt)	ND (0.03 ppt)
1,2,3,7,8,9-HxCDF	ND (0.09 ppt)	ND (0.09 ppt)	ND (0.11 ppt)
1,2,3,4,6,7,8-HpCDF	ND (0.04 ppt)	0.05	ND (0.55 ppt)
1,2,3,4,7,8,9-HpCDF	ND (0.04 ppt)	ND (0.04 ppt)	ND (0.29 ppt)
OCDF	ND (0.15 ppt)	ND (0.15 ppt)	ND (3.03 ppt)

Table 8. PCB Analysis Results for Four Lots of 18/12 TG

PCB	Concentration			
	Lot 2837	Lot 3813	Lot 4050	Lot 4098
IUPAC 28	NDR (76.7 ppt)	40.5 ppt	ND (8.7 ppt)	ND (7.4 ppt)
IUPAC 52	68.2 ppt	NDR (89.1 ppt)	20.1 ppt	ND (9.3 ppt)
IUPAC 77	42.7 ppt	76.8 ppt	NDR (11.9 ppt)	ND (10.5 ppt)
IUPAC 81	13.3 ppt	ND (13.3 ppt)	ND (8.1 ppt)	ND (8.7 ppt)
IUPAC 101	271 ppt	613 ppt	108 ppt	19.8 ppt
IUPAC 105	321 ppt	722 ppt	135 ppt	35.1 ppt
IUPAC 114	19.1 ppt	NDR (31.6 ppt)	ND (4.3 ppt)	ND (6.9 ppt)
IUPAC 118	537 ppt	1260 ppt	233 ppt	68.6 ppt
IUPAC 123	21.5 ppt	NDR (28.4 ppt)	NDR (11.1 ppt)	ND (7.3 ppt)
IUPAC 126	22.5 ppt	ND (10.1 ppt)	5.8 ppt	ND (6.1 ppt)
IUPAC 138	729 ppt	1520 ppt	480 ppt	120 ppt
IUPAC 153	500 ppt	1080 ppt	370 ppt	93.0 ppt
IUPAC 156	122 ppt	236 ppt	58.0 ppt	27.1 ppt
IUPAC 157	43.1 ppt	64.9 ppt	17.7 ppt	9.2 ppt
IUPAC 167	193 ppt	307 ppt	94.7 ppt	26.5 ppt
IUPAC 169	ND (8.6 ppt)	ND (10.1 ppt)	ND (9.1 ppt)	ND (6.7 ppt)
IUPAC 179	NDR (19.8 ppt)	32.6 ppt	NDR (12.9 ppt)	ND (6.1 ppt)
IUPAC 180	313 ppt	619 ppt	259 ppt	80.8 ppt
IUPAC 189	NDR (33.8 ppt)	NDR (20.1 ppt)	NDR (10.8 ppt)	ND (9.6 ppt)
IUPAC 190	N/Av	N/Av	N/Av	N/Av
Total PCBs	**0.006104 ppm**	**0.010754 ppm**	**0.003151 ppm**	**0.000681 ppm**

Table 9. Polycyclic Aromatic Hydrocarbon (PAH) Analysis Results for Four Lots of 18/12 TG

PAH	Concentration			
	Lot 2837	Lot 3813	Lot 4050	Lot 4098
Tetralin	6.5 ppb	6.3 ppb	8.5 ppb	9.6 ppb
Naphthalene	62.6 ppb	42.3 ppb	61.7 ppb	56.7 ppb
Quinoline	5.8 ppb	ND (0.9 ppb)	5.2 ppb	4.9 ppb
2-Methylnaphthalene	45.8 ppb	40.3 ppb	1.6 ppb	40.4 ppb
1-Methylnaphthalene	22.1 ppb	22.1 ppb	20.4 ppb	18.1 ppb
Biphenyl	13.9 ppb	15.0 ppb	12.5 ppb	14.3 ppb
2-Chloronaphthalene	ND (0.2 ppb)	ND (0.9 ppb)	ND (0.2 ppb)	ND (0.2 ppb)
2,6 & 2,7-dimethylnaphthalene	54.4 ppb	N/Av	30.2 ppb	31.7 ppb
1,2-dimethylnaphthalene	5.3 ppb	N/Av	5.4 ppb	6.1 ppb
Acenaphthylene	1.9 ppb	ND (0.5 ppb)	2.3 ppb	2.7 ppb
Acenaphthene	2.5 ppb	3.0 ppb	2.5 ppb	2.5 ppb
Fluorene	4.3 ppb	6.7 ppb	3.5 ppb	3.2 ppb
Phenanthrene	27.1 ppb	16.1 ppb	22.3 ppb	22.0 ppb
Anthracene	3.2 ppb	1.2 ppb	3.2 ppb	3.1 ppb
o-Terphenyl	ND (0.4 ppb)	ND (0.8 ppb)	ND (0.3 ppb)	ND (0.3 ppb)
m-Terphenyl	1.5 ppb	3.0 ppb	ND (1.0 ppb)	3.2 ppb
p-Terphenyl	ND (0.2 ppb)	ND (0.6 ppb)	ND (1.0 ppb)	ND (0.2 ppb)
2-Methylanthracene	3.3 ppb	2.6 ppb	1.7 ppb	3.1 ppb
9-Methylphenanthrene	4.2 ppb	ND (0.6 ppb)	4.3 ppb	4.2 ppb
1-Methylphenanthrene	6.9 ppb	3.3 ppb	12.4 ppb	6.0 ppb
Fluoranthene	9.9 ppb	1.5 ppb	26.4 ppb	9.9 ppb
Pyrene	11.3 ppb	10.3 ppb	32.1 ppb	11.2 ppb
9,10-Dimethylanthracene	ND (0.4 ppb)	9.5 ppb	ND (1.7 ppb)	ND (0.3 ppb)
Benzo(a)fluorene	2.6 ppb	ND (1.0 ppb)	22.3 ppb	1.7 ppb
Benzo(b)fluorene	5.8 ppb	ND (1.2 ppb)	17.8 ppb	1.2 ppb
Benz(a)anthracene	6.4 ppb	ND (0.7 ppb)	ND (1.4 ppb)	4.1 ppb
Triphenylene/Chrysene	6.3 ppb	ND (0.5 ppb)	ND (1.0 ppb)	6.2 ppb
Benz[b]anthracene	6.9 ppb	N/Av	ND (1.8 ppb)	ND (0.2 ppb)
7,12-dimethylbenz[a]anthracene	ND (0.6 ppb)	ND (1.7 ppb)	ND (4.2 ppb)	ND (0.5 ppb)
Benzo(b)fluoranthene	3.3 ppb	ND (0.3 ppb)	3.7 ppb	2.4 ppb
Benzo(k)&(j)fluoranthene	3.3 ppb	ND (0.5 ppb)	3.7 ppb	2.3 ppb
Benzo(e)pyrene	3.5 ppb	1.6 ppb	4.6 ppb	4.5 ppb
Benzo(a)pyrene	4.8 ppb	3.6 ppb	7.5 ppb	6.1 ppb
Perylene	ND (0.5 ppb)	ND (0.5 ppb)	ND (0.6 ppb)	ND (0.5 ppb)
3-Methylcholanthrene	ND (0.5 ppb)	ND (1.0 ppb)	ND (0.6 ppb)	ND (0.5 ppb)
Indeno-1,2,3(c,d)pyrene	ND (0.2 ppb)	ND (0.1 ppb)	ND (0.3 ppb)	ND (0.2 ppb)
Benzo(g,h,i)perylene	7.6 ppb	2.0 ppb	6.6 ppb	14.3 ppb

Table 9. Polycyclic Aromatic Hydrocarbon (PAH) Analysis Results for Four Lots of 18/12 TG

PAH	Concentration			
	Lot 2837	Lot 3813	Lot 4050	Lot 4098
Dibenz(ac)&(ah)anthracene	ND (0.3 ppb)	ND (0.2 ppb)	ND (0.5 ppb)	ND (0.3 ppb)
Picene	ND (0.4 ppb)	ND (0.2 ppb)	ND (0.6 ppb)	ND (0.4 ppb)
Coronene	ND (0.5 ppb)	ND (0.3 ppb)	ND (0.7 ppb)	ND (0.5 ppb)
Dibenz[a,e]pyrene	ND (0.8 ppb)	N/Av	ND (1.3 ppb)	ND (0.8 ppb)
Dibenz[a,i]pyrene	ND (1.9 ppb)	N/Av	ND (3.1 ppb)	ND (2.0 ppb)

G. Production Process for Microencapsulated Fish Oil

1. Encapsulated Loading

The encapsulated loading is 18/12 TG oil, produced and meeting specifications and purity standards as described previously. The production process by which microcapsules are formed around the oil is described in the following paragraphs and pictured schematically in Figure 3.

2. Slurry Preparation

Gelatin dry powder is dispersed in cold water to form a suspension. The gelatin used is Type A Pig Skin Gelatin meeting specifications for food grade material; no bovine-origin materials are used. The suspension is then warmed and stirred. Once the gelatin has been completely dissolved, the oil to be encapsulated (18/12 TG) is added and immediately homogenized to form an oil-in-water emulsion; homogenization continues until the desired average particle size is obtained.

Once the desired emulsion is achieved, pre-heated deionized water is added to reduce the gelatin concentration to an optimum level for coacervation. Agitation is adjusted to an optimum level for the increased volume and for coacervation and multicore particle formation. A prepared sodium polyphosphate solution is added to produce a partial dehydration/desolvation of the gelatin molecules at a temperature above the gelling point. The coacervation is initiated by adding phosphoric acid to the batch to reduce the pH. This causes the gelatin and polyphosphate to form coacervates and encapsulate the small emulsified oil particles. Acid addition is stopped once the desired particle size is achieved.

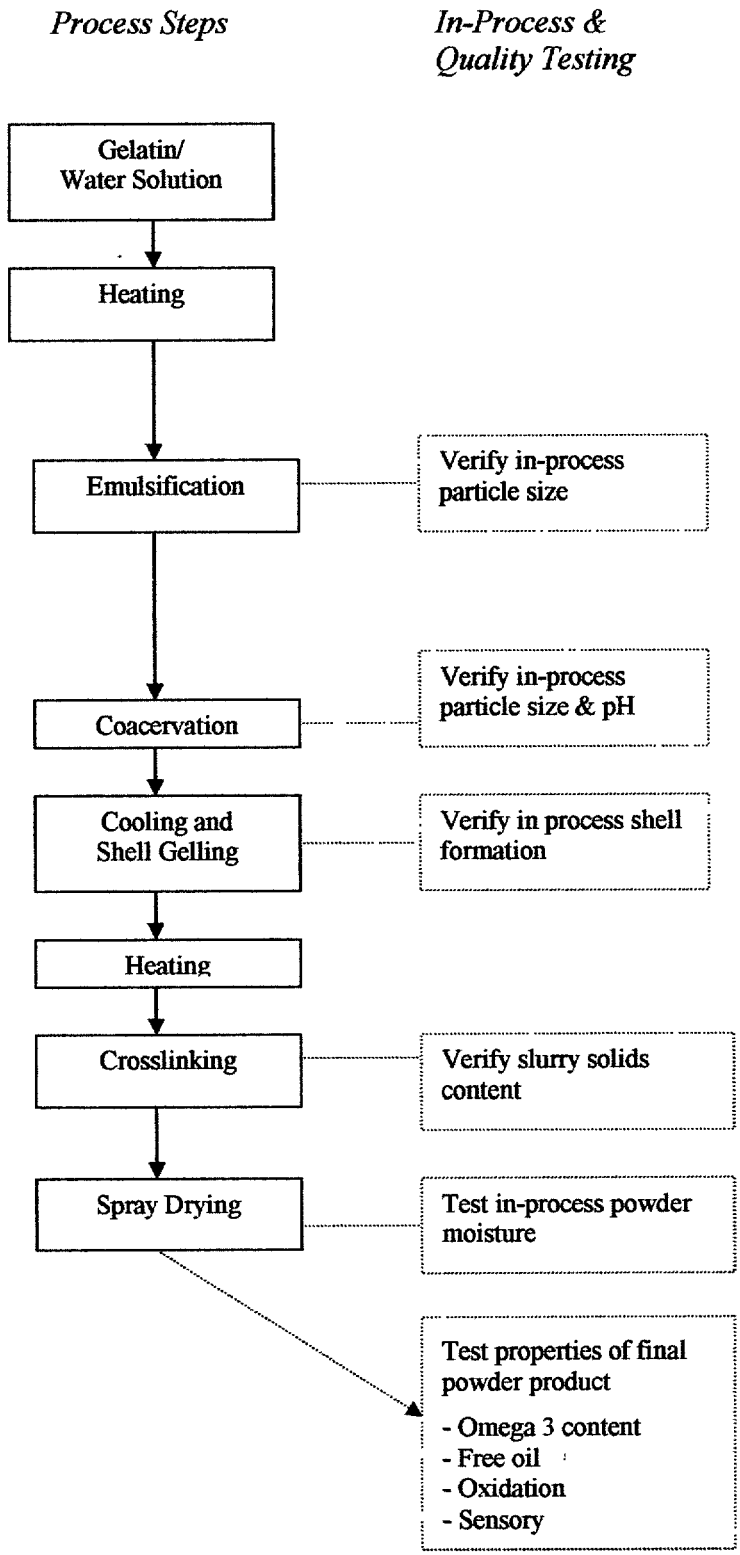
The batch is then cooled at a predetermined cooling rate so that the remaining shell materials in solution form shells around each of the microcapsules. This is continued until all free shell material (or free coacervates) has been consumed. The slurry is reheated and a cross-linking agent suitable for food use is added to strengthen the shell.

3. Spray Drying

The multicore microcapsule slurry is spray dried at optimum temperatures and feed rates to obtain a dry powder with desired moisture level and flow characteristics.

The product is tested to assure that it meets established specifications, including core loading, EPA and DHA content, average particle size, and maximum content of moisture and free (i.e., unmicroencapsulated) oil.

Figure 3. Flow Diagram of the Production Process for Microencapsulated Fish Oil



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H. Product Characteristics of Microencapsulated Fish Oil

1. Specifications for Food-Grade Microencapsulated Fish Oil

ONC has developed specifications for microencapsulated fish oil to demonstrate that it is food grade. These specifications are listed below, along with the reference to the ONC SOP for determining compliance with each specification (except for total PCBs, microbiological and metals, which are analyzed by a third-party contract laboratory using accepted validated methods for these contaminants).

• Appearance:	Free-flowing pale-cream colored powder (SOP 85/05/906)
• Odor and taste:	Bland odor, no fishy flavor (SOP 85/05/906)
• EPA content:	NLT 54 and NMT 114 mg/g of powder (SOP 85/05/917)
• DHA content:	NLT 42 and NMT 114 mg/g of powder (SOP 85/05/917)
• EPA+DHA content:	NLT 126 and NMT 204 mg/g of powder
• Average particle size:	NLT 35 and NMT 100 microns average (SOP 41/27/501)
• Free oil:	NMT 0.2% of powder weight (SOP 41/27/901)
• Moisture:	NMT 3.0% of powder weight (SOP 40/05/008)
• Core loading:	NLT 55% of powder weight (SOP 40/05/907)
• Sodium:	NMT 1.5% of powder weight (3rd Party Testing)
• Calcium:	NMT 0.37% of powder weight (3rd Party Testing)
• *Salmonella*:	Negative (3rd Party Testing)
• *E. coli*:	Negative (3rd Party Testing)
• *S. aureas*:	Negative (3rd Party Testing)
• Pseudomonas:	Negative (3rd Party Testing)
• Bacteria (ACC):	NMT 3000 CFU/g (3rd Party Testing)
• Yeast/mold:	NMT 300 CFU/g (3rd Party Testing)
• Coliform:	NMT 10 MPN/g (3rd Party Testing)
• Total PCBs:	NMT 0.09 ppm (3rd Party Testing)
• Arsenic (As):	NMT 3.5 ppm (3rd Party Testing)
• Lead (Pb):	NMT 0.5 ppm (3rd Party Testing)
• Mercury (Hg):	NMT 0.05 ppm (3rd Party Testing)
• Cadmium (Cd):	NMT 0.2 ppm (3rd Party Testing)

2. Batch Analysis Results

To demonstrate conformance with the proposed specifications listed above, ONC analyzed five batches or lots of microencapsulated fish oil. The results of these analyses are displayed in Table 10. These results show that all five lots of the final product are in full compliance with the established specifications, and thus the production process is under control.

Table 10. Batch Analysis Results for Five Lots of ONC's Microencapsulated Fish Oil						
Parameter	**Specification**	**Lot 5959**	**Lot 5957A**	**Lot A1315**	**Lot A1271**	**Lot A1437**
Appearance	Free-flowing pale-cream colored powder	Clumpy, peach	Fine, yellow	Fine, free-flowing light peach	Fine, off-white	Clumpy, yellow
Odor and Taste	Bland odor, no fishy flavor	Sweet green odor, soapy taste	Dry, slight sour odor, sour taste	Sour cream odor, sour cream taste	Slight sour cream odor, slight salty/ soapy taste	Dry, slight musty, slight sweet odor, salty, slight fishy taste
EPA content (mg/g)	54–114	91	94	96	89	92
DHA content (mg/g)	42–81	66	68	73	63	66
EPA+DHA content (mg/g)	126–204	157	162	169	152	158
Average Particle Size (microns)	35–100	56	67	55	72	61
Free Oil (%)	NMT 0.2	0.09	0.16	0.00	<0.005	0.04
Moisture (%)	NMT 3.0	1.8	2.0	2.5	2.7	2.0
Core Loading (%)	NLT 55	61	62	63	58	61
Sodium (%)	NMT 1.5	1.0	0.9%	1.2	1.1	0.91
Calcium (%)	NMT 0.37	0.038	0.01%	0.3	0.34	0.13
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative
E. coli	Negative	Negative	Negative	Negative	Negative	Negative
S. aureas	Negative	Negative	Negative	Negative	Negative	Negative
Pseudomonas	Negative	Negative	Negative	Negative	Negative	Negative
Bacteria (ACC) CFU/g	NMT 3000	<10	< 10	<10	<10	<10
Yeast/Mold CFU/g	NMT 300	<10	<10	<10	<10	<10
Coliform MPN/g	NMT 10	<3	<3	<3	<3	<3
Total PCBs (ppm)	NMT 0.09	0.02	0.02	0.02	0.02	0.02
Arsenic (ppm)	NMT 3.5	<0.1	<0.1	<0.1	<0.1	<0.1
Lead (ppm)	NMT 0.5	<0.1	<0.1	<0.1	<0.1	<0.1
Mercury (ppm)	NMT 0.05	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (ppm)	NMT 0.2	0.06	0.04	0.06	0.04	0.06

I. Analytical Method

The proposed analytical method for measuring the EPA and DHA content of marine oils and the foods to which these omega-3 fatty acids may be added is the EP 2003:1352 Method 2.4.29, slightly modified to an in-house method This method is designed to determine the fatty acid composition of marine oils in relative (area %) values, and EPA and DHA in absolute (mg/g) values using a bonded polyglycol liquid phase in a flexible fused silica capillary column. The method is applicable to the analysis of marine oils, capsules of EPA and DHA, and minor naturally occurring polyunsaturated fatty acids.

In the case of microcapsules, it is necessary to extract the oil prior to applying the method described above. The powder is physically ground with ethyl acetate in a nitrogen-flushed bowl of a planetary ball mill (Fritsch Pulverisette 6, 5 grinding balls at 400 rpm for 10 minutes). The oil is quantitatively extracted from the bowl by rinsing with hexane and filtering out the shell material. This procedure has been shown to produce accurate and reproducible results that agree closely with other techniques (enzymatic digestion of the shells, protein content, total fat analysis by acid digestion, SFE and ASE extraction methods).

III. REVIEW OF SAFETY DATA

A. Introduction

The FDA reviewed the safety of consumption of fish oil containing EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS under specified conditions of use (FDA 1997b). According to FDA, the primary safety concerns associated with excessive intakes of EPA and DHA include increased bleeding times, reduced glycemic control among diabetics, and increased levels of low-density lipoprotein (LDL) cholesterol among diabetics and hyperglycemics. ONC has expanded upon FDA's evaluation and reviewed the more recent literature to determine if more current information pertaining to these safety concerns would contradict what was concluded and recommended in the 1997 FDA opinion regarding EPA and DHA intake from fish oil. ONC commissioned a review by ENVIRON International Corporation in 2001 and commissioned JHEIMBACH LLC to update this review in 2003. This review has focused on the safety of fish oil and of intake of EPA+DHA combined rather than on the distinct metabolic effects of EPA and DHA in isolation.

In a letter addressing omega-3 fatty acid intake and health claims regarding coronary heart disease, FDA raised the issue of whether excessive EPA and DHA intakes may exert immunosuppressive effects (FDA 2000). Because of this concern raised by FDA, ONC also commissioned ENVIRON and JHEIMBACH to conduct independent reviews of the published scientific literature regarding the effects of fish oils containing EPA and DHA on the immune system.

B. Increased Bleeding Time

Evidence suggests that EPA and DHA may increase bleeding time, specifically by reducing platelet aggregability. Prolonged bleeding times in humans whose diets were supplemented with fish oil have been observed. However, the increases in bleeding time observed were not clinically significant; i.e., outside of the normal range for healthy adults, which is usually regarded as 1-9 minutes (Henry 1996). Therefore, uncertainty exists regarding the clinical relevance of these increased bleeding times following fish oil supplementation in the diet (Rodgers and Levin 1990). Other studies reporting increased bleeding times following daily intakes of more than 3 g/person/day of EPA and DHA used small numbers of test subjects (Atkinson et al. 1987; Harris et al. 1990; Jensen et al. 1988; Lorenz et al. 1983; Owens et al. 1990), making meaningful evaluation and interpretation of the results difficult. In addition, some of the studies that have used large numbers of healthy subjects did not observe statistically significant increases in bleeding time following daily intakes of EPA and DHA in amounts up to 3 g/person/day (Agren et al. 1990; Blonk et al. 1990; Desylpere et al. 1992; and Rogers et al. 1987).

As indicated above, some studies have shown statistically significant increases in bleeding time in subjects who consumed fish oils containing EPA and DHA (Sanders et al. 1981, 1983; Mortenson et al. 1983; Fischer and Weber, 1984; Thorngren et al. 1984; Knapp et al. 1986; Schmidt et al. 1990, 1992; Wojenski et al. 1991; Goodnight et al. 1981; Zucker et al. 1998; Harris et al. 1991). However, it should be noted that the observed increases were still within the

normal range reported for healthy adults. For example, healthy volunteers who supplemented their diet with 150 g of fatty fish per day for 12 weeks had a statistically significant increase in bleeding time compared to baseline (Thorngren et al. 1984). Similarly, healthy volunteers who consumed 1 pound/day of salmon (approximately 10 g/day of omega-3 fatty acids) had statistically significant prolonged bleeding times (Goodnight et al. 1981). In this study, the mean bleeding time prior to omega-3 fatty acid exposure was 6.75 minutes and increased to 10 minutes after 4 weeks of fish consumption. In another study, 20 healthy males consumed 4 g/day of EPA and DHA in fish oil capsules for 4 weeks (Mortensen et al. 1983). A small (16%) but statistically significant increase in bleeding time was observed relative to the control group, which received only vegetable oil. In yet another study, Harris et al. (1991) reported that daily consumption of fish oil containing 2.2 g of EPA and DHA produced a 15% increase in bleeding time that was statistically significantly elevated compared to baseline. However, this observed increase was still within the normal range of bleeding times for healthy adults.

In some studies, daily doses of fish oil resulted in prolonged bleeding times, as indicated by standard deviations of the mean bleeding times that fell outside of the baseline range, but statistical significance was not achieved. In one study (Grundt et al. 1999), healthy volunteers who supplemented their diets with fish oil containing 3.4 g EPA and DHA had increased bleeding times at the end of a 12-week study. The bleeding times increased by 30 seconds in the subjects who consumed fish oil, but this increase was not significant. In another study (Freese and Mutanen 1997), healthy subjects took fish oil capsules (containing 5.2 g of EPA and DHA) each day for 4 weeks followed by a 12-week follow-up period. There was an 18.5% increase in bleeding time in the fish oil group that returned to baseline during the follow-up period; however, no discussion or results of statistical analyses were provided.

Bleeding time data are also available from studies that have evaluated the effects of EPA and DHA intake on subjects having CHD or risk factors for CHD as their primary objective. In these studies, increased bleeding times were reported after daily intakes of EPA and DHA ranging from 3.2 to 6 g/day (Zucker et al. 1988; DeCaterina et al. 1990; Green et al. 1985; Smith et al. 1989; Schmidt et al. 1989; Solomon et al. 1990; Harris et al. 1991). However, the investigators did not discuss the clinical significance of these findings. For the most part, in studies in which fish oils were given to angioplasty or bypass surgery patients, excessive or prolonged bleeding times were not observed even though acetylsalicylic acid, a drug known to increase bleeding time, was used concurrently (Nilsen et al. 1991; Franzen et al. 1993; Bowles et al. 1991; Bairati et al. 1992; Grigg et al. 1989; Milner et al. 1989; Reis et al, 1989). In one study (Reis et al. 1989), an intake of 6 g/day of EPA and DHA in fish oils resulted in increased bleeding times in four out of 124 treated individuals (3%) as compared to controls. However, this observed increase was not statistically significant. In another study (Dehmer et al. 1988), patients who had undergone angioplasty received fish oil containing 5.4 g EPA and DHA daily for 9 months. In both the control and fish oil groups, the mean bleeding times were prolonged, but the increases were not statistically significant, and the reported bleeding times varied considerably between individuals. Heller et al. (2002) found no clinically significant differences in platelet function or coagulation after 6 days of daily administration of 86 mg/kg BW (6.0 g/70-kg person) EPA+DHA to patients recovering from major abdominal surgery. Eritsland et al. (1995) assigned 511 patients with CHD to either a fish oil group receiving 3.32 g of EPA and DHA per day, or to a control group. At the end of 9 months, bleeding time increased, but not significantly, in both the fish oil and the control groups, but the pre- and post-study bleeding time values were

within the normal range. In a similar study conducted by the same group, 610 patients admitted for coronary artery bypass grafting were given 3.32 g/day of EPA and DHA in addition to aspirin or warfarin for one year (Eritsland et al. 1996). In this study, there was a statistically significant increase in bleeding times in both groups at the end of the study compared to baseline, but there was no difference in bleeding times between the two groups.

The totality of the evidence found in the published scientific literature, including the more recent literature reviewed by ENVIRON and JHEIMBACH, demonstrates that when consumption of EPA and DHA is limited to 3 g/person/day or less, there is not a significant risk for increased bleeding time above the reported normal range. However, EPA and DHA intake exceeding 3 g/day may significantly prolong bleeding times in some individuals. Currently, there are insufficient data to evaluate the clinical significance of this phenomenon in these more highly sensitive individuals.

C. Reduced Glycemic Control

Studies on non-insulin-dependent diabetics have reported increased glucose levels when 4.5 to 8 g/day of EPA and DHA were added to the diet. Previously, the FDA addressed the possible adverse effects of fish oil on glycemic control in diabetics and stated that such effects were a safety concern (FDA 1993b). Also, the FDA established, based on review of several studies, that changes in blood glucose were dependent on the amount of fish oils (i.e., EPA and DHA) consumed (FDA 1993b).

One study (Annuzzi et al. 1991) failed to observe a change in blood glucose levels among type 2 (non-insulin-dependent) diabetics who ingested 3 g/day of EPA and DHA for 2 weeks as compared to those ingesting equal quantities of other fats. In addition, in two other studies (Hendra et al. 1990; Kasim et al. 1988) in which 3 g/day of EPA and DHA were administered for 6 weeks and 3 g of EPA and DHA for 8 weeks, respectively, only transient increases in blood glucose midway through the respective study periods were observed. In another study (Borkman et al. 1989), EPA and DHA administered in the diet for 3 weeks at 3 g/person/day caused comparable increases in fasting blood glucose when either fish or safflower oil was administered, with no significant difference between groups. Vessby and Boberg (1990) examined the effect of fish oil (3 g/day of EPA and DHA) and olive oil on glucose levels. In those who consumed fish oil, there was no change in fasting glucose or glycosylated hemoglobin levels compared to baseline. However, the olive oil supplementation appeared to increase glucose levels. In a study in non-insulin dependent diabetics (McGrath et al. 1996), the results showed that fish oil supplementation (3 g/day of EPA and DHA) increased fasting glucose concentrations; however, this increase was not statistically significant. Finally, studies in type 2 diabetics (Friday et al. 1989; Glauber et al. 1988; Schectman et al. 1988; Zambon et al. 1992) reported increased glucose levels, but subjects consumed relatively high levels (4.5 to 8 g/day) of EPA and DHA.

Farmer et al. (2001) conducted a meta-analysis of 18 published randomized placebo-controlled clinical trials of the effects of fish oil supplementation in type 2 diabetes, including 823 subjects followed for a mean of 12 weeks. The doses of fish oil administered ranged from 3 to 18 g/day; the intakes of EPA+DHA were from 1.7 to 10.0 g/day. Twelve studies reported fasting glucose levels and 11 reported glycosylated hemoglobin data in ways that permitted pooling of data;

none of these studies found significant changes nor was a significant effect found in the meta-analysis.

Yam et al. (2001) found no increase in glucose or evidence of hyperglycemia in hyperlipidemic subjects given 4.55 g/day EPA+DHA. In a study of hyperlipidemic patients taking simvastatin (Durrington et al. 2001), some of them also diabetic, no effect was noted on glycemic control in response to daily administration of 3.2 g EPA+DHA. Kesavulu et al (2002) found no change in glycemic control among non-insulin dependent diabetic patients receiving supplementation with 1.8 g/day EPA+DHA. Finally, in a study of obese men with dyslipidemia and insulin resistance, Chan et al. (2002) found no change in insulin resistance or in fasting blood glucose as a result of dosing with 3.36 g/day EPA+DHA in fish oil.

The FDA previously determined that EPA and DHA intakes of 3 g/person/day by diabetics exerted no clinically significant effect on glycemic control, although amounts in excess of 3 g/day may be a safety concern. Evaluation by ENVIRON and JHEIMBACH of more recent data studying the effects of fish oil containing EPA and DHA on glycemic control indicates that there is no new evidence to suggest that EPA and DHA at concentrations less than 3 g/day will adversely effect glycemic control in diabetics.

D. Increased LDL Cholesterol

The FDA noted that several studies in hypertriglyceridemic or hypercholesterolemic subjects reported increases in LDL cholesterol or apo B (apolipoprotein B, a major component of LDL) following fish oil consumption (FDA 1993b). Because elevated LDL cholesterol is a risk factor for CHD, the FDA re-evaluated these studies. As a result of this re-evaluation, the FDA found that although the available study conclusions are variable, there appears to be a trend toward increased LDL cholesterol corresponding with increased fish oil consumption in several population subgroups. The magnitude of the increase appears greater in populations with abnormal blood lipid levels, hypertension, diabetes, and cardiovascular disease. The FDA notes, however, that because the observations of increased LDL occurred in studies where large amounts of fish oils were given (resulting in EPA and DHA intakes of more than 5 g/day), any concern about changes in LDL cholesterol could be adequately addressed by limiting the intake of EPA and DHA to less than 3 g/person/day (FDA 1993b).

There has been considerable literature addressing this concern since the FDA review, but there is no indication that a change in FDA's conclusion is warranted. As discussed above, Farmer et al. (2001) conducted a meta-analysis of 18 published randomized placebo-controlled clinical trials of the effects of fish oil supplementation in type 2 diabetes. The studies included 823 subjects followed for a mean of 12 weeks, with doses of fish oil ranging from 3 to 18 g/day; the EPA+DHA intakes were from 1.7 to 10.0 g/day. Ten studies reported data on levels of LDL cholesterol. Only one study found a significant LDL-raising effect of EPA+DHA; the daily dose of EPA+DHA in this study was 6 g.

In a meta-analysis of randomized controlled trials of CHD patients, Bucher et al. (2002) reported 5 studies that studied the effects of dosing with EPA+DHA on LDL cholesterol. In 2 studies, with doses of 1.7 and 9.0 g/day EPA+DHA, LDL cholesterol increased by 7% and 5%, respectively; in 3 studies, with doses of 0.9, 4.8, and 6.9 g/day EPA+DHA, LDL cholesterol

decreased by 9%, 8%, and 6%, respectively. The authors concluded that intake of n-3 fatty acids within the levels tested has little effect on LDL levels.

Donadio et al. (2001) found no change in LDL cholesterol in patients with elevated creatine levels and IgA nephropathy dosed with 3.35 g/day EPA+DHA, and a significant decrease in LDL cholesterol for those given 6.70 g/day EPA+DHA. In a study of both normotriglyceridemic and hypertriglyceridemic subjects (Mabile et al. 2001), LDL cholesterol levels did not increase after 8 weeks of dosing with 3.0 g/day EPA+DHA.

Yam et al. (2001) found no increase in total or LDL cholesterol in hyperlipidemic subjects given 4.55 g/day EPA+DHA. In a study of hyperlipidemic patients taking simvastatin (Durrington et al. 2001), some of them also diabetic, no increase occurred in LDL cholesterol among either diabetics or non-diabetics in response to daily administration of 3.2 g EPA+DHA.

Nestel et al. (2002) found no effect on either total or LDL cholesterol due to dosing dyslipidemic subjects with 3.04 g/day EPA or 2.84 g DHA + 0.52 g DPA/day. Leigh-Firbank et al. (2002) found increases in LDL cholesterol and *ex vivo* LDL oxidation and a significant decrease in the percentage of LDL cholesterol as LDL_3 in response to a daily dose of 3.0 g EPA+DHA to mildly hypertriacylglycerolemic men. Puiggros et al. (2002) administered 2.45 g/day n-3 PUFA to hypercholesterolemic patients already consuming a diet rich in olive oil; no increase occurred in LDL cholesterol, although the level of lipoperoxides in isolated LDL showed a significant increase.

In the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) study, Marchioli et al. (2002) found that administration of 1.0 g/day EPA+DHA to patients with recent myocardial infarction caused an initial rise in LDL cholesterol levels to a maximum at approximately 6 months, followed by a return to baseline that was maintained to the latest measures at 42 months. This is particularly important, because it indicates that increase in LDL cholesterol in response to administration of EPA and DHA may in some cases be a temporary, reversible effect.

This review confirms that the totality of the published literature continues to be consistent with FDA's 1997 determination that intakes of EPA+DHA of 3 g/day or less do not adversely influence levels of LDL cholesterol.

E. Immunosuppressive Effects

Because EPA and DHA are known to alter several aspects of the human immune response (Calder 1998; Meydani et al. 1993), ONC asked ENVIRON to review this issue in 2001 and JHEIMBACH to update it in 2003. Data supporting this observation come in large part from *in vitro* studies evaluating immune cell function following dietary administration of fish oils to humans. Although discrete *in vitro* assays of cell function may be relevant indicators of the potential immune response against invading pathogens, the clinical significance of these data is unknown. To determine whether fish oil consumption is clinically immunosuppressive, the overall immune status of individuals consuming EPA- and DHA-containing fish oil must be considered. However, to date no study has established a relationship between fish oil intake and an increased incidence of disease or infection. Regardless, it is often difficult to establish a meaningful relationship between increased susceptibility to disease and exposure to a certain

chemical. The ability to attribute alterations in the immune system to a particular agent is made difficult by confounding factors such as lifestyle and diet, overall health status of the individual (e.g., presence of asthma or autoimmune disorders), and medications taken by the individual.

There is, however, a body of evidence suggesting that the immunosuppressive aspects of fish oil are beneficial for patients suffering from inflammatory diseases. Several studies in humans demonstrate that fish oil can ameliorate conditions of autoimmune and inflammatory diseases such as arthritis and ulcerative colitis (Kremer et al. 1987, 1995; Stenson et al. 1992; Almallah et al. 2000). In these cases, the beneficial effect of fish oil is attributed to the suppression of inflammation by reducing cellular activity and production of pro-inflammatory mediators that contribute to disease pathology.

In a double-blind, placebo-controlled study (Kremer et al. 1987), patients with rheumatoid arthritis consumed up to 6.3 g of EPA and DHA in fish oil on a daily basis for 14 weeks. Among the patients who consumed fish oil, there was a significant reduction in the number of swollen joints as compared to controls. Also, there was a significant reduction in levels of the inflammatory mediators leukotriene B4 (LTB4) and interleukin 1 (IL-1) that correlated with the reduction in swollen joints over time. In a similar study (Kremer et al. 1995), patients with rheumatoid arthritis received 9.75 g of omega-3 fatty acid supplements on a daily basis. The patients showed significant decreases in the number of tender joints, morning stiffness, and pain. Also, there was a significant decrease in circulating levels of IL-1 in patients consuming fish oil. However, there was a significant increase in the pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α). A significant increase in TNF-α was also observed in the control group. In another study, patients with ulcerative colitis received 5.4 g of EPA and DHA per day in fish oil for 16 weeks (Stenson et al. 1992). Fish oil supplementation resulted in a significant decrease in rectal dialysate levels of LTB4 and significant improvements in acute histology index and total histology index. The results of this study may, however, be due in part to the fact that the patients received concurrent treatment with prednisone. Similarly, patients with ulcerative colitis received 15 ml of fish oil extract containing 5.6 g of EPA and DHA daily for 6 months (Almallah et al. 2000). Results showed an improvement in disease activity accompanied by a decrease in serum levels of LTB4 and IL-2, and a decrease in natural killer (NK) cell activity among the patients who received fish oil. As with the Stenson et al. (1992) study, the patients maintained their existing anti-inflammatory medications during the course of the study, making it difficult to solely attribute the improvement in disease parameters to fish oil. The authors concluded that although the modest clinical improvement does not support a recommendation for fish oil supplementation as a single treatment for acute ulcerative colitis, it could be a useful co-treatment to reduce the dosage of steroids or anti-inflammatory medications required.

Researchers have also examined the effect of fish oil supplementation on other diseases mediated by inflammation, including asthma and psoriasis (Ziboh et al. 1986; Bittiner et al. 1988). The results of these studies do not provide convincing evidence that fish oil is beneficial for treatment of these diseases.

Fish oil has also been shown to decrease fever in humans, another component of the inflammatory response. Healthy volunteers who supplemented their daily diet with fish oil containing 2.71 g of EPA and DHA for 8 weeks were challenged with typhoid vaccine (Cooper et al.1993). The fish oil inhibited an expected rise in oral temperature following vaccination;

however, this effect was not statistically significant relative to the control group. *In vitro* stimulation of lymphocytes obtained from the individuals who consumed fish oil showed decreased production of IL-1, a cytokine associated with the induction of fever.

One study (Kelley et al. 1998) demonstrated that DHA (in the absence of EPA) caused a decrease in the number of circulating white blood cells (WBCs) in healthy males who supplemented their diets with moderately high levels of DHA (6 g/day) for 90 days. There was a significant decrease in the number of WBCs at day 113 in the DHA group, as compared to controls. The decrease in WBCs was primarily due to a 21% decrease in the number of polymorphonuclear cells (PMNs). Although the number of PMNs was significantly decreased in the DHA group, the residual counts were still within clinically normal ranges. This same study also examined the effect of DHA on the delayed hypersensitivity response to antigen, an *in vivo* test of immune function. There was no significant difference between the DHA group and controls in their ability to elicit an antigenic response following sensitization. Also, DHA did not appear to influence IL-2 production or mitogen-stimulated lymphocyte proliferation, which was actually increased in the DHA group.

Donadio et al. (2001) administered doses of 6.70 g and 3.35 g EPA+DHA to patients with IgA nephropathy; there were no unfavorable effects on peripheral blood leukocytes. In a complex study, Thies et al. (2001a, 2001b) supplemented the diets of healthy adults age 55–75 with oils providing daily intakes of 2000 mg α-linolenic acid (ALNA), 770 mg γ-linolenic acid (GLA), 680 mg arachidonic acid (ARA), 720 mg DHA, or 1000 mg EPA+DHA (720 mg EPA + 280 mg DHA) for 12 weeks. The biological markers measured were lymphocyte proliferation; production of interleukin-2 (IL-2) and interferon-γ (IFN-γ); the number and proportion of T and B lymphocytes, helper and cytotoxic T lymphocytes, memory helper T lymphocytes, leukocytes, and NK cells in the circulation; proportion of leukocytes as total lymphocytes and proportion of lymphocytes as T lymphocytes and as NK cells; and NK cell activity. The only markers that showed significant differences among the groups, or changes from baseline, were lymphocyte proliferation (reduced by GLA and by EPA+DHA) and NK cell activity (reduced by EPA+DHA).

Turini et al. (2001) administered 25 g/day fish oil (comprising 4.3 g EPA + 2.8 g DHA) to healthy men and found no decrease in immune cell activities; on the contrary, an increase in phagocytic activity was seen in blood monocytes.

In a study of patients recovering from major abdominal surgery (Weiss et al. 2002), administration of 4.3 g/day EPA+DHA resulted in a decrease in levels of IL-6, but no change in TNF-α and an increase in monocytic human leucocyte antigen (HLA)-DR, a marker of immune competence. There was no difference between the test and control groups in the number of infections, although the n-3 fatty acid group's infections were less severe than were those that occurred in the control group. Schauder et al. (2002) also used postoperative abdominal surgery patients to study the effect of 86 mg/kg BW/day (5.8 g/day for mean=68-kg person) EPA+DHA on a variety of immune markers. No significant effects were found other than potentially beneficial increases in several markers (IL-2, IFN-γ, and TNF-α); the authors concluded that fish oil at the dosage given is not immunosuppressive in moderately stressed surgical patients.

A majority of the existing studies that have examined the effect of EPA and DHA intake on the immune system have done so by assessing discrete cellular functions in healthy human volunteers following fish oil supplementation. For the most part, daily supplementation with fish oil (EPA and DHA) resulted in decreased or altered immune cell functions including expression of cell surface molecules, cytokine secretion, and proliferation in response to mitogen stimulation. However, it should be noted that the *in vivo* cells of the immune system exist as part of a network influenced by other cell types. Therefore, the purification and isolation of the particular cell types to be studied often disrupts or alters such interactions, and interpretation of these types of results, including trying to determine their clinical relevance, can be difficult.

The effect of EPA and DHA intake on cell surface marker expression was examined when healthy volunteers supplemented their diets with 1.56 g EPA and DHA per day for 3 weeks (Hughes et al. 1996). There was a decreased level of major histocompatibility complex class II (MHC class II) expression on the surface of peripheral blood monocytes. The expression of MHC molecules on cell surfaces is required for antigen presentation during the initiation of the immune response. Although caution should be exercised in interpreting the results of this study, which involved a small number of participants (6), the findings support the possibility that fish oil may be beneficial in the treatment of autoimmune disorders, which are associated with the abnormally elevated expression of both MHC class II molecules.

Several studies have found changes in macrophage and monocyte-derived cytokine production (Endres et al. 1989; Meydani et al. 1991, 1993; Cooper et al. 1993) following fish oil consumption. Peripheral blood monocytes from healthy adults consuming 1.23 to 6 g of fish oil per day for 6 to 24 weeks produced less TNF-α and IL-1 than controls. Similarly, 4 weeks of supplementation of healthy volunteers with linseed or fish oil (2.7 g EPA and DHA per day) demonstrated slightly lower *ex vivo* production of TNF-αand IL-1, with the effect of fish oil being more pronounced (Caughey et al. 1996). In another study, healthy males supplemented their diet with 6 g DHA per day for 90 days (Kelley et al. 1999). Here, DHA significantly decreased IL-1, TNF-α, and PGE_2 production by stimulated peripheral blood mononuclear cells. NK cell activity in the DHA group was also significantly decreased compared to controls. Healthy males receiving DHA capsules only (6 g/day) did not demonstrate a decrease in NK cell activity (Thies et al. 2001a), although in the same study, a group that consumed fish oil containing both EPA and DHA (9 g/day) did show a significant decrease in NK activity after 12 weeks.

In addition, it has been demonstrated that fish oil diets alter lymphocyte-derived cytokine production as well. For example, supplementation of the diet of healthy volunteers with 1.23 or 2.4 g/day of EPA and DHA for 12 or 24 weeks lowered *ex vivo* IL-2 production (Meydani et al. 1991). Also, lymphocytes isolated from volunteers who supplemented their diet with 18 g of marine lipids (4.6 g EPA and DHA) per day for 6 weeks exhibited a significant decrease in IL-2 production (Endres et al. 1993). This was paralleled by a marked decrease in mitogen-induced proliferation, a functional parameter closely associated with IL-2 production. Interestingly, maximal suppression of IL-2 production was observed as late as 10 weeks after cessation of the fish oil supplementation. This is consistent with a previous study by Endres et al. (1989) that demonstrated significant decreases in TNF-α and IL-1 production as late as 10 weeks after supplementation with 4.55 g of EPA and DHA per day was discontinued. This phenomenon

may be a result of re-utilization of n-3 fatty acids from a slow-turnover compartment of fatty acids.

Decreased lymphocyte proliferation in response to antigenic stimulation following fish oil consumption has been reported by several investigators (Meydani 1991; Meydani and Dinarello 1993; Kremer et al. 1987; Santoli and Zurier 1989; Kelly et al. 1991). However, Payan and Goetzl (1983) found an increase in lymphocyte proliferation in asthma patients and Kremer et al. (1987) observed an increase in lymphocyte proliferation in patients with rheumatoid arthritis after fish oil consumption. The conflicting observations might be due to differences in health status, length of dietary treatment, drug use, patient age, and reagents used for the *in vitro* studies. Several immune parameters were examined in volunteers who supplemented their diets with daily doses of 2.4 g of EPA for 12 weeks (Virella et al. 1991). No consistent changes were observed in neutrophil function tests, mitogen-stimulated lymphocyte proliferation, and immunoglobulin and antibody synthesis. Production of IL-2 from lymphocytes following stimulation appeared to be decreased, although this effect was not significant.

Some studies have focused on the effect of fish oils on neutrophil function. Neutrophils are essential to host defenses against pathogens, although neutrophils often contribute to the pathology of several inflammatory disorders, in large part through the production of superoxide radicals. Healy et al. (2000) examined the effect of fish oil supplementation (up to 4 g of EPA and DHA per day) on neutrophil function in healthy volunteers after 12 weeks. Neutrophil fatty acid composition changed, but there was no difference in superoxide generation or chemotaxis compared to controls. Neutrophil functions have only been reported to be attenuated when the combined intakes of EPA and DHA were well above 5 g/day (Luostarinen et al. 1996; Sperling et al. 1993).

Although there are no human data describing a relationship between fish oil intake and decreased host defenses, a study in mice identified an effect of dietary fish oil on bacterial clearance (Fritsche et al. 1997). Female C3H mice were given special diets supplemented with menhaden fish oil (16% EPA and 12% DHA), lard or soybean oil. After 4 weeks of consuming the experimental diet, the mice were challenged with a sublethal dose of *Listeria monocytogenes*, an intracellular bacterium that has been used extensively as a model with which to study host resistance. The mice fed the fish oil demonstrated decreased bacterial clearance 4 days post-infection as compared to controls. These results are in contrast with those reported by Rubin et al. (1989). This study also examined the effect of dietary fish oil on the response to *Listeria* and failed to show an effect. This may be due to the strains of mice that were used for the different studies. Rubin et al. used NZB autoimmune-disease prone mice, which may be more resistant to *Listeria* than the C3H strain. This discrepancy underscores the uncertainty associated with establishing a relationship between a certain chemical and alterations in host resistance, even when using animal models.

One issue that was not raised by FDA is the potential for lactating women consuming fish oil supplements to exhibit an adverse effect on the immune components in their milk, with possible impact on the immune status of the nursing infant. Hawkes and her colleagues (2001, 2002) explored the question of whether dietary supplementation of lactating women with EPA and DHA can modulate the concentration of cytokines in the aqueous phase of human milk and the production of cytokines by human milk cells. In addition to a placebo group, they administered

tuna oil containing either 370 mg EPA+DHA (70 mg EPA, 300 mg DHA) or 740 mg EPA+DHA (140 mg EPA, 600 mg DHA) daily to healthy post-partum lactating women for 4 weeks. While this supplementation did increase the n-3 PUFA concentrations in relevant tissues, it did not perturb the cytokine concentrations (IL-1β, IL-6, TNF-α, transforming growth factor-β1 (TGF-β1), and TGF-β2) in the milk.

It is difficult to assign clinical relevance to discrete alterations within the immune system. The cytokine network of the immune system is varied and redundant, e.g., several cytokines have the same effect on the same cell population. Therefore, one is unable to conclude that decreased cytokine production is an indicator of overall decreased host resistance following dietary administration of EPA and DHA. Similarly, *ex vivo* lymphocyte proliferation in response to mitogen is not reflective of *in vivo* conditions. In the host, lymphocyte proliferation following antigenic challenge is a multi-stage process that involves many cell types, and it is difficult to interpret these observations without considering overall host resistance to disease and infection. It should be noted, however, that the authors of the previously described studies in which test subjects consumed EPA- and DHA-containing fish oil did not describe any increased incidence of disease or infection. In addition, it is unclear from evidence generated in the animal studies described above whether or not EPA and DHA disrupt host resistance against pathogens. Furthermore, no human studies exist that support the hypothesis that consumption of fish oil causes increased susceptibility to infection and disease.

The results of some clinical studies have suggested a beneficial effect of fish oil (EPA and DHA) consumption on diseases or conditions associated with inflammation, but suppression or alteration of certain immune parameters could be significant, especially in those individuals with compromised immune systems. However, the EPA and DHA intakes at which significant alterations of immune function were observed were relatively high (4 to 9.75 g/person/day). Therefore, it is reasonable to suggest that consumption of fish oil resulting in EPA and DHA intakes of up to 3 g/day would not be expected to compromise overall host defenses, and thus would be protective of any potential immunosuppressive effects of EPA and DHA.

This conclusion is supported by the recent prepublication report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Macronutrients (FNB-IOM 2002). After reviewing the available literature regarding the impact of consumption of EPA and DHA on immune function, bleeding and increased risk of hemorrhagic stroke, and oxidative damage, the Committee concluded (pp. 8–57):

> While there is evidence to suggest that high intakes of n-3 polyunsaturated fatty acids, particularly EPA and DHA, may impair immune response and result in excessively prolonged bleeding times, it is not possible to establish a UL. Studies on immune function were done *in vitro* and it is difficult, if not impossible, to know how well these artificial conditions simulate human immune cell response *in vivo*. Data on EPA and DHA intakes and bleeding times are mixed and dose-response effect was not observed.

F. Contaminants and Residuals of Potential Toxicological Significance

1. Pesticides

Four lots of ONC's 18/12 TG were analyzed for pesticide residues, using highly sensitive analytical methods, and no residues were detected in any of the lots (Table 5). There is thus no basis for any concern with potential exposure to pesticide residues as a result of the proposed uses of 18/12 TG.

2. Dioxins and Furans

Polychlorinated dibenzo-p-dioxins (PCDDs or dioxins) and polychlorinated dibenzofurans (PCDFs or furans) are formed during chemical processes involving chlorine and certain organic chemicals and during exposure of chlorine-containing organic chemicals to high temperatures. Dioxins are present ubiquitously in the environment and in food, especially in animal fats.

In humans, observed adverse effects associated with dioxin exposure have been limited to highly exposed populations in occupational settings or following accidental exposures or contamination episodes. Some epidemiological studies suggest an association between chronic dioxin exposure and cancer, diabetes, liver, cardiovascular, and reproductive effects, immunosuppression, and various effects in children on development and thyroid hormone status. Conclusive evidence of adverse human health effects at these lower exposure levels is lacking, however, because of incomplete exposure data, concomitant exposure to other compounds, and small numbers of study participants. Furthermore, reported effects are not necessarily consistent across studies.

There are many individual chemical forms (congeners) of PCDDs and PCDFs differing in the number and/or positions of chlorine atoms attached to the basic ring systems. The congeners differ in their relative toxicities. ONC relied on the toxic equivalence (TEQ) procedure developed under the auspices of the North Atlantic Treaty Organization (NATO) and refined in 1997 by the World Health Organization (WHO; Van den Berg et al. 1998). The TEQ procedure assigns a toxic equivalency factor (TEF) to each PCDD or PCDF congener based on its toxicity relative to 2,3,7,8-TCDD, the most toxic congener. The TEQ concentration expressed in 2,3,7,8-TCDD toxic equivalents is derived by multiplying the concentration of each congener, by the corresponding TEF and summing the cross-products.

This TEF scheme was adopted by the FDA in its assessment of the potential health risks associated with dioxin exposure from bleached paper products (FDA 1990). FDA determined that exposure for a full lifetime to a dose of 2,3,7,8-TCDD of 0.064 pg/kg BW/day corresponds to an upper limit on cancer risk of one in 1,000,000; or 1×10^{-6}. This is equivalent to 3.8 pg/day for a 60-kg person.

Employing the TEQ approach, ONC estimated the exposure to dioxins and furans from the analytical results for the three lots of ONC's 18/12 TG shown in Tables 6 and 7, and the estimated potential intake of ONC's 18/12 TG of 10.0 g/person/day. All non-detects were set at one-half the detection limit. The table below shows the potential daily exposure to dioxins and furans, in TCDD equivalents, for each of the three tested lots of 18/12 TG.

Lot Number	Total TEQ (pg/g)	TCDD-Equivalent Intake (pg/day)
3813	0.132	1.32
4050	0.125	1.25
4098	0.130	1.30

The estimated TCDD-equivalent intakes for all three lots of ONC's 18/12 TG are well below 3.8 pg/day, with a mean estimated intake of 1.29 pg/day, and thus ONC's 18/12 TG does not present the potential for adverse health effects from exposure to dioxins and furans under its intended conditions of use.

3. PCBs

A number of PCB congeners have dioxin-like activity and these congeners have been assigned TEFs similar to those assigned to dioxins and furans. These TEFs were summed to derive a total TEQ for dioxin-like PCBs detected in each tested lot of 18/12 TG; those congeners that were not detected were assumed to be present at one-half the detection limit. The total TEQ concentration derived for each lot was multiplied by the estimated consumption of ONC's 18/12 TG of 10.0 g/person/day to arrive at an estimated exposure to dioxin-like PCBs from the proposed uses of ONC's 18/12 TG as shown in the table below.

Lot Number	Total PCB TEQ (pg/g)	TCDD-Equivalent Intake (pg/day)
2837	0.360	3.60
3813	0.268	2.68
4050	0.115	1.15
4098	0.084	0.84

The estimated TCDD-equivalent intakes for dioxin-like PCBs in all three lots of ONC's 18/12 TG are below 3.8 pg/day, with a mean estimated intake of 2.07 pg/day, and thus ONC's 18/12 TG does not present the potential for adverse health effects from exposure to dioxin-like PCBs under its intended conditions of use.

The mean combined TCDD-equivalent intake for all dioxin-like compounds (DLCs: dioxins, furans, and dioxin-like PCBs) is 3.36 pg/day, below 3.8 pg/day, and thus ONC's 18/12 TG does not present the potential for adverse health effects from exposure to DLCs under its intended conditions of use.

4. Polycyclic Aromatic Hydrocarbons (PAHs)

The PAH analysis results for the four lots of ONC's 18/12 TG showed detectable levels of a number of PAHs (Table 9). To evaluate the potential toxicological significance of these PAH levels, ONC calculated estimated exposures to all PAHs. The mean concentration of each PAH across the four tested lots was calculated; PAHs that were not detected were assumed to be

present at one-half the LOD. This mean concentration was multiplied by the estimated intake of 18/12 TG of 10 g/person/day.

The USEPA and the Agency for Toxic Substances and Disease Registry (ATSDR) have established reference doses (RfDs) or minimal risk levels (MRLs), respectively, for nine of the PAHs potentially present in 18/12 TG (USEPA 2002; ATSDR 2002). For two PAHs, cancer-risk slope factors have been estimated by USEPA (2002). Neither the USEPA nor the ATSDR has established acceptable exposure levels for the remaining PAHs. Therefore, the Threshold of Regulation (TOR) limit of 1.5E-3 mg/day established by FDA for indirect food additives was used as an estimate of acceptable intake. Because this TOR limit of 1.5E-3 mg/day is a toxicologically based value that describes an intake level for any noncarcinogenic substance below which there is no health or safety concern, it may be interpreted as an ADI that is appropriate for any noncarcinogenic substance for which no specific RfD or MRL has been established.

In Table 11, the EDI of each PAH potentially present in 18/12 TG is compared with its ADI. For ADIs expressed in mg/kg, the ADI given is for a 70-kg adult. For each ADI, the source and the basis are indicated: FDA's TOR value, the oral RfD established by USEPA, the oral MRL established by ATSDR, or the Cancer Slope Factor (CSF) established by USEPA. In no case does the EDI exceed the ADI, and in most cases the EDI is several orders of magnitude lower than the ADI. Margins of safety (MOS) were calculated and are presented in the final column of Table 11. The lowest MOS is 1.7 for benzo(a)pyrene; four others are less than 10; seventeen are between 10 and 100; and the remainder are greater than 100. Thus, there are no potential adverse effects from exposure to PAHs from 18/12 TG oil or microcapsules used as proposed.

Table 11. Margins of Safety (MOS) for Exposures to Polycyclic Aromatic Hydrocarbons (PAH) from ONC's 18/12 TG

PAH	PAH Concentrations (ppb)					EDI (mg)	ADI (mg)	Source of ADI	MOS
	Lot 2837	Lot 3813	Lot 4050	Lot 4098	Mean				
Tetralin	6.50	6.30	8.50	9.60	7.73	7.7E-05	1.5E-03	FDA TOR	19.4
Naphthalene	62.60	42.30	61.70	56.70	55.83	5.6E-04	1.4E-00	USEPA RfD	2507.8
Quinoline	5.80	0.45	5.20	4.90	4.09	4.1E-05	2.3E-04	USEPA CSF E-5	5.6
2-Methyl-naphthalene	45.80	40.30	1.60	40.40	32.03	3.2E-04	1.5E-03	FDA TOR	4.7
1-Methyl-naphthalene	22.10	22.10	20.40	18.10	20.68	2.1E-04	4.9E-00	ATSDR MRL	23700.1
Biphenyl	13.90	15.00	12.50	14.30	13.93	1.4E-04	3.5E-00	USEPA RfD	25134.6
2-Chloro-naphthalene	0.10	0.45	0.10	0.10	0.19	1.9E-06	5.6E-00	USEPA RfD	2986666.7
2,6 & 2,7-Dimethyl-naphthalene	54.40	0	30.20	31.70	29.08	2.9E-04	1.5E-03	FDA TOR	5.2
1,2-Dimethyl-naphthalene	5.30	0	5.40	6.10	4.20	4.2E-05	1.5E-03	FDA TOR	35.7
Acenaphthylene	1.90	0.25	2.30	2.70	1.79	1.8E-05	1.5E-03	FDA TOR	83.9
Acenaphthene	2.50	3.00	2.50	2.50	2.63	2.6E-05	4.2E-00	USEPA RfD	160000.0
Fluorene	4.30	6.70	3.50	3.20	4.43	4.4E-05	2.8E-00	USEPA RfD	63276.8
Phenanthrene	27.10	16.10	22.30	22.00	21.88	2.2E-04	1.5E-03	FDA TOR	6.9
Anthracene	3.20	1.20	3.20	3.10	2.68	2.7E-05	2.1E+01	USEPA RfD	785046.7
o-Terphenyl	0.20	0.40	0.15	0.15	0.23	2.3E-06	1.5E-03	FDA TOR	666.7
m-Terphenyl	1.50	3.00	0.05	3.20	1.94	1.9E-05	1.5E-03	FDA TOR	77.4
p-Terphenyl	0.10	0.30	0.50	0.10	0.25	2.5E-06	1.5E-03	FDA TOR	600.0
2-Methyl-anthracene	3.30	2.60	1.70	3.10	2.68	2.7E-05	1.5E-03	FDA TOR	56.1
9-Methyl-phenanthrene	4.20	0.30	4.30	4.20	3.25	3.3E-05	1.5E-03	FDA TOR	46.2
1-Methyl-phenanthrene	6.90	3.30	12.40	6.00	7.15	7.2E-05	1.5E-03	FDA TOR	21.0
Fluoranthene	9.90	1.50	26.40	9.90	11.93	1.2E-04	2.8E-00	USEPA RfD	23480.1
Pyrene	11.30	10.30	32.10	11.20	16.23	1.6E-04	3.0E-02	USEPA RfD	184.9
9,10-Dimethyl-anthracene	0.20	9.50	0.85	0.15	2.68	2.7E-05	1.5E-03	FDA TOR	56.1
Benzo(a)fluorene	2.60	0.50	22.30	1.70	6.78	6.8E-05	1.5E-03	FDA TOR	22.1
Benzo(b)fluorene	5.80	0.60	17.80	1.20	6.35	6.4E-05	1.5E-03	FDA TOR	23.6
Benz(a)anthracene	6.40	0.35	0.70	4.10	2.89	2.9E-05	1.5E-03	FDA TOR	51.9
Triphenylene/ Chrysene	6.30	0.25	0.50	6.20	3.31	3.3E-05	1.5E-03	FDA TOR	45.3
Benz[b]anthracene	6.90	0	0.90	0.10	1.98	2.0E-05	1.5E-03	FDA TOR	75.9

Table 11. Margins of Safety (MOS) for Exposures to Polycyclic Aromatic Hydrocarbons (PAH) from ONC's 18/12 TG

PAH	PAH Concentrations (ppb)					EDI (mg)	ADI (mg)	Source of ADI	MOS
	Lot 2837	Lot 3813	Lot 4050	Lot 4098	Mean				
7,12-Dimethyl-benz[a]anthracene	0.30	0.85	2.10	0.25	0.88	8.8E-06	1.5E-03	FDA TOR	171.4
Benzo(b)-fluoranthene	3.30	0.15	3.70	2.40	2.39	2.4E-05	1.5E-03	FDA TOR	62.8
Benzo(k)&(j)-fluoranthene	3.30	0.25	3.70	2.30	2.39	2.4E-05	1.5E-03	FDA TOR	62.8
Benzo(e)pyrene	3.50	1.60	4.60	4.50	3.55	3.6E-05	1.5E-03	FDA TOR	42.3
Benzo(a)pyrene	4.80	3.60	7.50	6.10	5.50	5.5E-05	9.6E-05	USEPA CSF E-5	1.7
Perylene	0.25	0.25	0.30	0.25	0.26	2.6E-06	1.5E-03	FDA TOR	571.4
3-Methyl-cholanthrene	0.25	0.50	0.30	0.25	0.33	3.3E-06	1.5E-03	FDA TOR	461.5
Indeno-1,2,3(c,d)pyrene	0.10	0.05	0.15	0.10	0.10	1.0E-06	1.5E-03	FDA TOR	1500.0
Benzo(g,h,i)-perylene	7.60	2.00	6.60	14.30	7.63	7.6E-05	1.5E-03	FDA TOR	19.7
Dibenz(ac)&(ah)-anthracene	0.15	0.10	0.25	0.15	0.16	1.6E-06	1.5E-03	FDA TOR	923.1
Picene	0.20	0.10	0.30	0.20	0.20	2.0E-06	1.5E-03	FDA TOR	750.0
Coronene	0.25	0.15	0.35	0.25	0.25	2.5E-06	1.5E-03	FDA TOR	600.0
Dibenz[a,e]pyrene	0.40	0	0.65	0.40	0.36	3.6E-06	1.5E-03	FDA TOR	413.8
Dibenz[a,i]pyrene	0.95	0	1.55	1.00	0.88	8.8E-06	1.5E-03	FDA TOR	171.4

IV. SAFETY ASSESSMENT/GRAS DETERMINATION

A. Introduction

This chapter presents an assessment that demonstrates that 18/12 TG, in both oil and powder form, is safe, and is also GRAS under the FDCA for direct addition to foods as a nutrient supplement at specified use levels in a variety of foods, as listed in Table 2. This safety assessment and GRAS determination entails two steps. In step one, the safety of 18/12 TG under its intended conditions of use is demonstrated. Safety is established by showing that 18/12 TG is substantially equivalent to edible fish oils and fish oils already regarded as GRAS for addition to foods and comparing the EDIs of EPA+DHA and all impurities from 18/12 TG under its intended conditions of use with the ADIs for EPA+DHA and for all impurities. A substance directly added to food is considered safe for its intended use if the EDI of the substance under its intended conditions of use is less than or approximates its ADI (FDA 1993a). In the second step, 18/12 TG is determined to be GRAS by demonstrating that the safety of this substance under its intended conditions of use is generally recognized among qualified scientific experts.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FDCA is set forth under 21 CFR 170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under 21 CFR 170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under 21 CFR 170.30(c). This GRAS determination employs scientific procedures established under 21 CFR 170.30(b).

A key concept is that of substantial equivalence. In its proposed rule establishing the GRAS notification process (FDA 1997a), FDA cited the recommendations of a 1996 joint consultation by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). This consultation (FAO/WHO 1996) recommended that, "if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e. the food or food component can be concluded to be as safe as the conventional food or food component). Account should be taken of any processing that the food or food component may undergo as well as the intended use and the intake by the population."

In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether 18/12 TG, employed as a nutrient supplement, is safe and is also GRAS for the uses and at the use levels shown in Table 2. Once 18/12 TG, both oil and powder

(microcapsules), is determined to be GRAS for its intended uses, it is permitted to be used for those purposes because it is not (by definition) a food additive, and therefore does not require promulgation of a food additive regulation under 21 CFR prior to being lawfully marketed and sold in the U.S.

B. Safety of 18/12 TG Oil and Microcapsules

A scientific procedures GRAS determination requires first that information about the substance establish that the intended use of the substance is safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR 170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

1) The probable consumption of the substance and of any substance formed in or on food because of its use (i.e., the EDI);

2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet; and

3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

After consideration of these factors, an ADI and an EDI are typically derived for the substance. The ADI represents the maximum amount of the substance that has been shown to be safe for consumption by humans on a daily basis for a lifetime. An EDI for the substance is derived based on the probable human consumption of the substance and of any substance formed in or on food because of its use. Finally, the EDI for a substance is compared against its ADI. As long as the EDI is less than or approximates the ADI, the substance can be considered safe for its intended use (FDA 1993a).

As was noted earlier, 18/12 TG is substantially equivalent to other edible fish oils and also fish oils, such as menhaden oil and small planktivorous pelagic fish body oil (SPPFBO), that are already GRAS for addition to foods (FDA 1997b, FDA 2002c). The primary distinguishing characteristic of fish oils as compared with other oils used in foods is their higher content of the omega-3 fatty acids EPA and DHA. ONC's 18/12 TG contains, on average, about 30% EPA+DHA; SPPFBO also contains about 30% EPA+DHA while menhaden oil contains about 20%. The ratio of EPA:DHA in the three oils is similar: the ratio in 18/12 TG and in SPPFBO is about 1.5:1 and the ratio in menhaden oil is about 1.7:1. Since 18/12 TG is substantially equivalent to edible fish oils as well as these GRAS oils, it can be concluded to be as safe as they are, subject to considerations of the intended use and the intake by the population and processing to remove impurities.

1. EDI of EPA and DHA

As indicated above, 21 CFR 170.3(i) requires that, in evaluating the safety of the proposed use of a new food additive, the probable consumption (i.e., the EDI) of the substance and of any

substance formed in or on food because of its use be considered, as well as the cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet. Thus, because a scientific procedures GRAS determination requires the same quantity and quality of evidence as is required to obtain approval of the substance as a new food additive, a scientific procedures GRAS determination must also consider the probable consumption and cumulative effect of the substance in the diet.

The estimated mean potential intake of EPA+DHA from 18/12 TG from all proposed use categories by users of one or more foods is 3 g/day. The estimated mean potential intake of 18/12 TG oil is 10 g/day and the estimated mean potential intake of 18/12 TG powder (microcapsules) is 18 g/day. These figures are based on an average EPA+DHA content of 30% in the oil and 17% in the powder, which is 55–60% oil.

Results of the estimates of exposure from all food categories indicate that more than 99% of the U.S. population consume one or more of the foods and beverages included in the list of proposed uses over a nonconsecutive two-day period. The calculated EDIs are likely considerable overestimates of exposure to 18/12 TG, as these estimates assume that all foods in the proposed use categories contain 18/12 TG at the maximum proposed use levels. Intake of EPA+DHA from 18/12 TG does not increase the potential intake over that already provided by menhaden oil, SPPFBO, and other GRAS fish oils because all fish-oil products are alternatives that are used under the same restrictions regarding EPA+DHA addition. The cumulative EDI of EPA and DHA from both naturally occurring sources (0.1 g/person) and the proposed uses of 18/12 TG oil or microcapsules (3 g/person) is, therefore, about 3.1 g/person.

2. ADI for EPA and DHA

The FDA has previously reviewed safety concerns regarding consumption of fish oil containing the two omega-3 fatty acids EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS (FDA 1997b). The primary safety concerns evaluated by the FDA associated with excessive intakes of EPA and DHA included increased bleeding times, reduced glycemic control among diabetics, and increased levels of LDL cholesterol among diabetics and hyperglycemics. Based on this review, the FDA concluded that a combined intake of EPA and DHA of up to 3 g/person/day would not result in any adverse health effects. As described in Chapter III, two evaluations (by ENVIRON in 2001 and by JHEIMBACH in 2003) were conducted of the more recently published scientific literature to determine if there is any new information pertaining to the FDA's safety concerns that would contradict what was concluded and recommended by FDA in the 1997 review of EPA and DHA intake from fish oil. In addition, as was also described in Chapter III, because of a more recent concern raised by FDA regarding potential immunosuppressive effects of EPA and DHA, a review was conducted of the published literature relevant to the effects of EPA and DHA on the immune system. These reviews focused on studies of fish oils and of EPA and DHA in combination, rather than on the distinct metabolic effects of EPA and DHA ingested independently.

Based on the review presented in Chapter III, the following conclusions were reached regarding a safe level of intake of EPA and DHA from consumption of fish oil:

- The totality of the evidence found in the published scientific literature demonstrates that, when consumption of EPA and DHA is limited to 3 g/person/day or less, there is no significant risk for increased bleeding time above the reported normal range.

- Evaluation of more recent data on the effects of fish oil containing EPA and DHA on glycemic control indicates that there is no new evidence to suggest that EPA and DHA intakes of less than 3 g/day will adversely affect glycemic control in diabetics.

- An examination of the recent literature regarding the effect of EPA- and DHA-containing fish oil on LDL is consistent with the conclusion reached by the FDA that daily consumption of fish oils resulting in intakes of EPA and DHA less than 3 g/day does not adversely influence LDL levels.

- Consumption of fish oil resulting in EPA and DHA intakes of up to 3 g/day would not be expected to compromise overall host defenses, and thus would be protective of any potential immunosuppressive effects of EPA and DHA.

Therefore, the more recent literature is consistent with FDA's determination that the ADI for EPA and DHA combined from the consumption of fish oil is 3 g/person/day, and that this ADI can be used to evaluate the safety of 18/12 TG for direct addition to food. The evidence is less clear with respect to the independent effects of EPA and DHA, but there is nothing in the literature that would indicate a potential hazard due to intakes of either fatty acid in the range of 1–2 g/person/day with an intake of the two combined not exceeding 3 g/person/day.

3. Establishing the Safety of 18/12 TG Oil and Microcapsules

As a result of the proposed uses and use level of 18/12 TG oil and microcapsules, the EDI of EPA+DHA is about 3 g/person/day. To this EDI of EPA and DHA from 18/12 TG, the potential contribution of EPA and DHA from naturally occurring sources must be added. EPA and DHA from these natural sources could contribute as much as 0.1 g/person/day, yielding a cumulative EDI of 3.1 g per person.

The cumulative EDI of EPA and DHA of 3.1 g/person/day, due to the addition of 18/12 TG oil or microcapsules to various foods at the proposed use levels and intake from naturally occurring sources, approximates the ADI for EPA and DHA of 3 g/day established by the FDA and confirmed by an assessment of research published since the FDA review. Furthermore, the EDIs of all impurities in 18/12 TG oil are less than their respective ADIs. Thus, the proposed uses and use levels of 18/12 TG can be considered safe.

C. General Recognition of the Safety of 18/12 TG Oil and Microcapsules

The proposed uses and use level of 18/12 TG have been determined to be safe through scientific procedures set forth under 21 CFR 170.30(b). This safety was established by demonstrating that 18/12 TG is substantially equivalent to other fish oils already GRAS for addition to foods, followed by estimating potential human exposure to EPA and DHA from the intended uses of 18/12 TG. Next, the FDA's determination that an intake of EPA and DHA of up to 3 g/person/day from consumption of menhaden oil is safe was employed to establish an ADI for EPA+DHA of 3 g/person/day. The FDA scientists who participated in this determination are

considered to be experts qualified by scientific training and experience to evaluate safe levels of exposure to EPA and DHA. The published scientific literature on which the FDA's safe intake level was based was updated and reviewed to confirm that the ADI for EPA and DHA established by FDA is consistent with current information. Then, the probable human exposure, or EDI, for EPA and DHA, resulting from the proposed uses and use level of 18/12 TG in food, was compared to the ADI for EPA and DHA. Because the EDI approximates the ADI, and EDIs for no impurities exceed their respective ADIs, 18/12 TG can be considered safe for its intended uses at the specified use levels. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of 18/12 TG for direct addition to foods in either oil or powder form under the intended conditions of use has been made through the deliberations of Robert G. Ackman, Ph.D., Joseph F. Borzelleca, Ph.D., and Walter H. Glinsmann, M.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, and have concluded:

> *ONC's 18/12 TG is substantially equivalent to other fish oils that are already GRAS for addition to foods. No evidence exists in the available information on EPA and DHA that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when EPA and DHA are used at levels that are now current or that might reasonably be expected from the proposed uses of ONC's 18/12 TG oil or powder as a nutrient supplement.*

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, 18/12 TG is safe and is GRAS for the proposed uses described in Table 2. Because ONC's fish oil is GRAS for its proposed uses, it is excluded from the definition of a food additive, and thus may be lawfully marketed and sold for these uses in the U.S. without the promulgation of a food additive regulation under 21 CFR.

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AM

Hendrickson, Carrie

From:

Sent: Tuesday, December 16, 2003 4:28 PM

To: Hendrickson, Carrie

Subject: Re: GRN 138-fish oil

Dear Carrie--

Sorry for the slow response; I was traveling.

ND = Non-Detect. It means that the substance is present, if at all, below the value at which it could be detected with the analytical method employed. (The number in parenthesis is the limit of detection (LOD), which may vary from sample to sample for a number of reasons: instrument noise, matrix effects and interferences, variability with the extraction procedure, and day-to-day fluctuations in instrument response.)

NDR = Non-Detect Resolution. It means that a peak was detected, but did not meet quantification criteria. It is equivalent to the limit of quantitation (LOQ). (The number in parenthesis is the LOQ, which may vary for the same reasons as the LOD.)

N/Av = Not Available.

Hope this answers your questions. Please let me know if additional questions arise.

Jim

James T. Heimbach, Ph.D., F.A.C.N.
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April 2, 2004

Linda S. Kahl, Ph.D.
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Linda:

This is an amendment to GRAS notice GRN 138, originally filed on October 3, 2003.

As required, three copies are provided.

If you have any questions regarding this amendment, please feel free to contact me at 202-237-8406 or jim@jheimbach.com.

Sincerely

James T. Heimbach, Ph.D., F.A.C.N.

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GRN 138 (October 3, 2003)
AMENDMENT

This amendment to GRN 138 provides for the use of activated carbon on a regular basis in the production of 18/12 TG Fish Oil and Fish Oil Microcapsules.

As the production process was described in the original notice, activated carbon could optionally be used along with bleaching clay in the bleaching step of the production process. (This was shown in Figure 2, "Generalized Schematic of the Production Process for 18/12 TG," on page 11 of the notice.) This amendment removes the word "optional" and requires that activated carbon be used as a standard part of the production of 18/12 TG fish oil products.

Ocean Nutrition Canada, Ltd., (ONC) is making this change in order to reduce the concentration of polyaromatic hydrocarbons (PAHs) in the product to assure that consumers receive a food-grade product of high quality and assured purity.

BACKGROUND

Pollution of ocean waters with crude petroleum is a worldwide phenomenon. Although most media attention focuses on oil spills, in fact more crude petroleum reaches the oceans from natural seeps and from the fuels and lubricants used by every ship (Robert G Ackman, Canadian Institute of Fisheries Technology, personal communication). Additional hydrocarbons reach the oceans from rivers and from precipitation. The gills in fish have the ocean water on one side and the fish blood supply on the other, primarily to extract oxygen from the water. Any chemicals in true solution pass through the gills from higher to lower concentration, and thus fish develop concentrations of these hydrocarbons, including polyaromatics.

NORIT Americas, Inc., a maker of activated carbon products, has analyzed the PAH content of crude oil derived from fish caught in the waters of North and South American, Scandinavia, and Japan, and has found concentrations of light PAHs (such as anthracene, phenanthrene, fluoranthene, and pyrene) exceeding 2000 µg/kg (parts per billion, ppb) and concentrations of heavy PAHs (such as benz(a)pyrene, benz(e)pyrene, and 1,12-benzperylene) approaching 1000 ppb. Data included by Norit in their sales literature are shown in Table 1, below.

Table 1. PAH Levels in Crude Fish Oils (NORIT Americas, Inc.)

		PAH in µg/kg (ppb)			
		Light[1]		Heavy[2]	
Crude Oil	Origin	Range	Median	Range	Median
Fish	North America	3 - 23	13	1 – 6	3
Fish	Scandinavia	38 - 109	85	5 – 8	6
Fish	South America	11 - 2383	486	1 – 149	29
Fish	Japan	17 - 1935	584	2 – 900	49

1. Anthracene
   Phenanthrene
   Fluoranthene
   Pyrene
   1,2-Benzanthracene
   Chrysene

2. Benz(a)pyrene
   Benz(e)pyrene
   Perylene
   Anthanthrene
   1,12-Benzperylene
   1,2,5,6-Dibenzanthracene
   Coronene

Additionally, in the UK Total Diet Study (TDS), PAH concentrations were measured in a variety of foods sampled in 2000. Non-zero levels of PAHs were found in all foods tested, most notably fatty foods such as nuts, fats and oils, and fish. Average PAH concentrations found in the UK TDS are shown in Table 2 of the December 2002 report, "PAHs in the UK Diet: 2000 Total Diet Study Samples," which is included with this amendment. It may be noted that in the UK TDS, the edible portion of fish was analyzed. Since PAHs are lipophilic, the concentrations of PAHs in oils extracted from these fish would undoubtedly be considerably higher than those found in the whole fish.

As would be expected, ONC's 18/12 TG fish oil products also contain low levels of PAHs. Despite the generally low levels, it is appropriate to further reduce PAHs to the lowest levels technically feasible without harming the nutritional value of the product.

ACTIVATED CARBON

Solids such as activated carbon (an amorphous form of carbon) and synthetic resins are widely used in industrial applications and for purification of waters and wastewaters. Activated carbon is an effective adsorbent primarily due to its extensive porosity and very large available surface area. The chemical nature of the carbon's adsorptive surface is also important. Activated carbon was first used to remove undesirable odors and tastes from drinking water, but in recent years the use of activated carbon for the removal of organic pollutants has become very common.

ONC has demonstrated that treatment of its fish-oil products with activated carbon during processing significantly reduces PAH concentrations. In Table 2, below, PAH reduction is shown in a bonito oil purchased by ONC from Japan, which in its crude form contained relatively high concentrations of PAHs.

Table 2. PAH Analysis of One Lot of ONC Bonito Oil Before and After Treatment with Activated Carbon

Test	Bonito Oil Lot #7470	
Light PAHs	**Before carbon**	**After carbon**
Benz(a)anthracene	160 ppb	**0.9 ppb**
Anthracene	70 ppb	**1.6 ppb**
Chrysene	180 ppb	**2.6 ppb**
Phenanthrene	395 ppb	**38 ppb**
Fluoranthene	400 ppb	**20 ppb**
Pyrene	385 ppb	**39 ppb**
Acenaphthene	62 ppb	**15 ppb**
Heavy PAHs		
Dibenz(a, h)anthracene	4.5 ppb	**ND (0.4 ppb)**
Benzo(a)pyrene	65 ppb	**0.2 ppb**
Benzo(b)fluoranthene	85 ppb	**0.4 ppb**
Benzo(k)fluoranthene	37 ppb	**0.1 ppb**
Indeno-1,2,3(c,d)pyrene	16 ppb	**ND (0.5 ppb)**
Benzo(g,h,i)perylene	14 ppb	**ND (0.4 ppb)**
Benzo(e)pyrene	N/Av ppb	**3.0 ppb**
Perylene	N/Av ppb	**ND (1.0 ppb)**
Anthanthrene	N/Av ppb	**ND (1.0 ppb)**
Coronene	N/Av ppb	**ND (1.0 ppb)**
Additional PAHs by GC-MS		
Naphthalene	71 ppb	**1.8 ppb**
2-Methylnaphthalene	65 ppb	**1.9 ppb**
2,6 & 2,7 - dimethylnaphthalene	15 ppb	**1.8 ppb**
1-Methylnaphthalene	70 ppb	**2.4 ppb**
1,2 - dimethylnaphthalene	5.4 ppb	**2.3 ppb**

As is evident, treatment with activated carbon reduced PAH concentrations by as much as three orders of magnitude.

In Table 3, below, are shown results of treatment of two lots of 18/12 TG oil with activated carbon. One lot had been previously alkali-refined (an optional processing step described in the GRAS notice) and the other had not. PAH concentrations in these oils prior to carbon treatment were far lower than they were in crude bonito oil, so the reduction is not as dramatic, but the final post-treatment PAH concentrations were all below the 1ppb or better limits of detection of the LC-Fluorescence method.

Table 3. PAH Analyses of Two Lots of ONC 18/12 TG Fish Oil Before and After Treatment with Activated Carbon

Test	18/12 TG Lot #3813		18/12 TG Alkali-refined Lot #7055	
Light PAHs	**Before carbon**	**After carbon**	**Before carbon**	**After carbon**
Benz(a)anthracene	ND (0.5 ppb)	**ND (0.5 ppb)**	ND (0.5 ppb)	**ND (0.5 ppb)**
Anthracene	ND (1.0 ppb)	**ND (1.0 ppb)**	ND (1.0 ppb)	**ND (1.0 ppb)**
Chrysene	ND (0.5 ppb)	**ND (0.5 ppb)**	ND (0.5 ppb)	**ND (0.5 ppb)**
Phenanthrene	ND (1.0 ppb)	**ND (1.0 ppb)**	9.4 ppb	**ND (1.0 ppb)**
Fluoranthene	ND (1.0 ppb)	**ND (1.0 ppb)**	1.3 ppb	**ND (1.0 ppb)**
Pyrene	ND (1.0 ppb)	**ND (1.0 ppb)**	2.6 ppb	**ND (1.0 ppb)**
Acenaphthene	ND (1.0 ppb)	**ND (1.0 ppb)**	2.7 ppb	**ND (1.0 ppb)**
Heavy PAHs				
Dibenz(a, h)anthracene	ND (0.4 ppb)	**ND (0.4 ppb)**	ND (0.4 ppb)	**ND (0.4 ppb)**
Benzo(a)pyrene	0.2 ppb	**ND (0.1 ppb)**	0.3 ppb	**ND (0.1 ppb)**
Benzo(b)fluoranthene	0.2 ppb	**ND (0.1 ppb)**	0.3 ppb	**ND (0.1 ppb)**
Benzo(k)fluoranthene	0.1 ppb	**ND (0.1 ppb)**	0.1 ppb	**ND (0.1 ppb)**
Indeno-1,2,3(c,d)pyrene	ND (0.5 ppb)	**ND (0.5 ppb)**	ND (0.5 ppb)	**ND (0.5 ppb)**
Benzo(g,h,i)perylene	ND (0.4 ppb)	**ND (0.4 ppb)**	ND (0.4 ppb)	**ND (0.4 ppb)**
Benzo(e)pyrene	ND (1.0 ppb)	**ND (1.0 ppb)**	ND (1.0 ppb)	**ND (1.0 ppb)**
Perylene	ND (1.0 ppb)	**ND (1.0 ppb)**	ND (1.0 ppb)	**ND (1.0 ppb)**
Anthanthrene	ND (1.0 ppb)	**ND (1.0 ppb)**	ND (1.0 ppb)	**ND (1.0 ppb)**
Coronene	ND (1.0 ppb)	**ND (1.0 ppb)**	ND (1.0 ppb)	**ND (1.0 ppb)**
Additional PAHs by GC-MS				
Naphthalene	<1.0 ppb	**<1.0 ppb**	1.8 ppb	**<1.0 ppb**
2-Methylnaphthalene	<1.0 ppb	**<1.0 ppb**	<1.0 ppb	**<1.0 ppb**
2,6 & 2,7 – dimethylnaphthalene	<1.0 ppb	**<1.0 ppb**	<1.0 ppb	**<1.0 ppb**
1-Methylnaphthalene	<1.0 ppb	**<1.0 ppb**	<1.0 ppb	**<1.0 ppb**
1,2 – dimethylnaphthalene	<1.0 ppb	**<1.0 ppb**	<1.0 ppb	**<1.0 ppb**

CONCLUSION

Treatment of ONC's 18/12 TG fish oil with activated carbon is an effective means of assuring that the product is low in PAHs. **This step has been incorporated as a standard part of the processing of this fish oil, and the GRAS notice is hereby amended to reflect this change.**

Table 2: Concentrations (ppb fresh weight) of PAHs in Total Diet Study samples in 2000

PAH	Abbrev.	COC rank	Concentrations (ppb fresh weight)									
			Bread	Misc cereals	Carcase meat	Offals	Meat products	Poultry	Fish	Oils and fats	Eggs	Sugars & preserves
benzo(a)pyrene	BaP	A	0.11	0.09	<0.04	<0.04	<0.04	<0.04	<0.08	0.45	<0.04	<0.08
benz (a) anthracene	BaA	A	0.10	0.08	<0.01	<0.01	0.03	0.01	0.08	0.28	<0.01	0.10
dibenz(ah)anthracene	DBahA	A	<0.01	<0.02	<0.01	<0.01	<0.03	<0.01	<0.01	<0.01	<0.02	<0.02
anthanthrene	Anth	B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
benzo(b)fluoranthene	BbFl	B	0.09	0.08	<0.04	<0.04	0.04	<0.04	0.10	0.39	<0.04	<0.08
benzo(k)fluoranthene	BkFl	B	<0.05	0.05	<0.04	<0.04	<0.04	<0.04	0.04	0.23	<0.04	<0.05
benzo(b)naphtho(2,1-d)thiophene	BNT	B	<0.01	0.04	<0.01	<0.01	0.02	<0.01	0.04	<0.01	<0.01	0.05
benzo(g,h,i) perylene	BghiPl	B	0.09	0.09	0.03	<0.01	0.04	<0.03	0.08	0.51	<0.01	0.06
chrysene	Chry	B	0.19	0.16	0.02	0.02	0.07	0.02	0.18	0.53	0.01	0.20
cyclopenta(c,d)pyrene	CPP	B	<0.01	0.05	<0.01	<0.01	0.02	<0.01	0.03	0.06	<0.01	0.04
indeno(1,2,3-cd) pyrene	IP	B	0.08	0.06	<0.04	<0.04	<0.04	<0.04	<0.06	0.39	<0.04	0.05
acenaphthylene	Acnl	D	0.17	0.13	0.08	0.06	0.21	0.08	0.97	0.28	0.02	0.16
acenaphthene	Acn	D	1.51	1.17	1.26	0.68	1.34	1.11	1.15	1.86	0.13	0.53
benzo(e)pyrene	BeP	D	0.09	0.07	<0.01	<0.01	0.03	<0.01	0.12	0.37	<0.01	0.06
fluorene	F	D	0.76	0.64	0.56	0.34	0.76	0.40	1.37	0.97	0.12	0.66
phenanthrene	Ph	E	1.98	2.31	0.79	0.57	1.12	0.64	2.33	2.80	0.23	4.97
anthracene	A	E	0.13	0.08	0.03	0.03	0.08	0.03	0.25	0.18	<0.01	0.25
fluoranthene	Fl	E	0.44	0.49	0.15	0.12	0.24	0.13	0.56	0.80	0.05	1.00
pyrene	P	E	0.49	0.51	0.11	0.10	0.23	0.12	0.50	0.92	0.05	0.89
***Upper bound* total**			**6.32**	**6.13**	**3.25**	**2.15**	**4.39**	**2.78**	**7.96**	**11.05**	**0.86**	**9.26**

Note: Concentrations in italics are indicative values due to co-elution with other PAHs.

Table 2 (*continued*): Concentrations (ppb fresh weight) of PAHs in Total Diet Study samples in 2000

PAH	Abbrev.	Concentrations (ppb fresh weight)									
		Green vegetables	Potatoes	Other vegetables	Canned vegetables	Fresh fruit	Fruit products	Beverages	Milk	Dairy products	Nuts
benzo(a)pyrene	BaP	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	0.06
benz (a) anthracene	BaA	0.04	0.01	0.03	0.01	0.01	0.01	<0.01	<0.01	0.02	0.09
dibenz(ah)anthracene	DBahA	<0.04	<0.02	<0.01	<0.01	<0.02	<0.02	<0.02	<0.01	<0.02	<0.02
anthanthrene	Anth	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
benzo(b)fluoranthene	BbFl	0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	0.07
benzo(k)fluoranthene	BkFl	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
benzo(b)naphtho(2,1-d)thiophene	BNT	0.02	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.19
benzo(g,h,i) perylene	BghiPl	<0.03	0.02	0.02	0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.06
chrysene	Chry	0.13	0.03	0.06	0.03	0.03	0.03	<0.01	0.01	0.04	0.30
cyclopenta(c,d)pyrene	CPP	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.04
indeno(1,2,3-cd) pyrene	IP	<0.03	<0.04	<0.03	<0.03	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
acenaphthylene	Acnl	0.05	0.02	0.04	0.03	0.02	0.01	<0.01	0.03	0.04	0.69
acenaphthene	Acn	<0.71	0.41	0.38	0.14	0.42	0.08	0.04	0.17	0.49	1.89
benzo(e)pyrene	BeP	0.05	0.02	0.02	0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.06
fluorene	F	<0.53	0.21	0.26	0.11	0.39	0.08	0.04	0.14	0.29	2.31
phenanthrene	Ph	1.18	0.37	0.69	0.31	1.46	0.22	0.08	0.32	0.55	7.69
anthracene	A	0.06	0.02	0.02	0.02	0.03	0.01	<0.01	<0.01	0.03	0.32
fluoranthene	Fl	0.42	0.08	0.17	0.08	0.17	0.06	0.02	0.09	0.17	0.67
pyrene	P	0.28	0.07	0.15	0.08	0.12	0.06	0.03	0.09	0.32	0.73
***Upper bound* total**		**3.71**	**1.47**	**2.03**	**1.02**	**2.88**	**0.79**	**0.48**	**1.09**	**2.21**	**15.28**

AM

Hendrickson, Carrie

From:

Sent: Tuesday, April 13, 2004 9:01 AM

To: Hendrickson, Carrie

Subject: GRN 138

Dear Carrie--

You have noted that the reported concentrations of polyaromatic hydrocarbons (PAHs) in Lot 3813 differ between the original GRAS notice and the amendment submitted on April 2, 2004. This difference is due to the fact that the two reports are based on analyses conducted by two different laboratories at two different times using two different methods.

Please call me if you have further questions.

Jim Heimbach

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